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Downregulation of muscarinic- and 5-HT_{1B}-mediated modulation of [^{3}H]acetylcholine release in hippocampal slices of rats with fimbria-fornix lesions and intrahippocampal grafts of septal origin

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Abstract

Adult Long-Evans female rats sustained electrolytic fimbria-fornix lesions and, two weeks later, received intrahippocampal suspension grafts of fetal septal tissue. Sham-operated and lesion-only rats served as controls. Between 6.5 and 8 months after grafting, both the [^{3}H]choline accumulation and the electrically evoked [^{3}H]acetylcholine ([^{3}H]ACh) release were assessed in hippocampal slices. The release of [^{3}H]ACh was measured in presence of atropine (muscarinic antagonist, 1 μM), physostigmine (acetylcholinesterase inhibitor, 0.1 μM), oxotremorine (muscarinic agonist, 0.01 μM–10 μM), mecamylamine (nicotinic antagonist, 10 μM), methiothepin (mixed 5-HT_1/5-HT_2 antagonist, 10 μM), 8-OH-DPAT (5-HT_{1A} agonist, 1 μM), 2-methyl-serotonin (5-HT_3 agonist, 1 μM) and CP 93129 (5-HT_{1B} agonist, 0.1 μM–100 μM), or without any drug application as a control. In lesion-only rats, the specific accumulation of [^{3}H]choline was reduced to 46% of normal and the release of [^{3}H]ACh to 32% (nCi) and 43% (% of tissue tritium content). In the grafted rats, these parameters were significantly increased to 63%, 98% and 116% of control, respectively. Physostigmine reduced the evoked [^{3}H]ACh release and was significantly more effective in grafted (−70%) than in sham-operated (−56%) or lesion-only (−54%) rats. When physostigmine was superfused throughout, mecamylamine had no effect. Conversely, atropine induced a significant increase of [^{3}H]ACh release in all groups, but this increase was significantly larger in sham-operated rats (+209%) than in the other groups (lesioned: +80%; grafted: +117%). Oxotremorine dose-dependently decreased the [^{3}H]ACh release, but in lesion-only rats, this effect was significantly lower than in sham-operated rats. Whatever group was considered, 8-OH-DPAT, methiothepin and 2-methyl-serotonin failed to induce any significant effect on [^{3}H]ACh release. In contrast, CP 93129 dose-dependently decreased [^{3}H]ACh release. This effect was significantly weaker in grafted rats than in the rats of the two other groups. Our data confirm that cholinergic terminals in the intact hippocampus possess inhibitory muscarinic autoreceptors and serotonin heteroreceptors of the 5-HT_{1B} subtype. They also show that both types of receptors are still operative in the cholinergic terminals which survived the lesions and in the grafted cholinergic neurons. However, the muscarinic receptors in both lesioned and grafted rats, as well as the 5-HT_{1B} receptors in grafted rats show a sensitivity which seems to be downregulated in comparison to that found in sham-operated rats. In the grafted rats, both types of downregulations might contribute to (or reflect) an increased cholinergic function that results from a reduction of the inhibitory tonus which ACh and serotonin exert at the level of the cholinergic terminal.

Keywords: Acetylcholine release; Atropine; CP 93129; Fimbria-fornix; Hippocampus; Mecamylamine; Methiothepin; 2-methyl-Serotonin; Oxotremorine; Septal graft

1. Introduction

Partial or extensive lesions of the septo-hippocampal pathways are considered as one of the possible experimental models of Alzheimer's disease in animals (e.g., [19,26]). Such lesions are known to induce a series of deficits including perturbations of the electrophysiological characteristics of normal hippocampal functioning (e.g., rhythmical slow wave activity and place fields; [8,48]), severe and lasting depletions of some hippocampal neurotransmitters and related enzymes or metabolites (e.g., acetylcholine (ACh), acetylcholinesterase (AChE), choline acetyltrans-

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ferase (ChAT) activity, serotonin; [13,14,18,22,27,29,30,52]), as well as dramatic impairments of learning and memory processes in which the hippocampus plays a major role (e.g., [11,20,21,34,43]). Some of these lesion-induced disturbances can be attenuated or even compensated for by intrahippocampal grafts rich in fetal cholinergic neurons [19]. The mechanisms by which these grafts exert functional effects can be multiple. As regards the possible compensation for depleted neurotransmitter systems in a given brain structure of the recipient, these mechanisms may involve (i) acute or chronic release of growth-promoting and/or neurotrophic factors, (ii) diffuse 'minipump-like' delivery of neurotransmitters into the brain parenchyma and/or the ventricles, and (iii) target-directed release of neurotransmitters within specific newly established synaptic contacts between grafted neurons and neurons located in the host structure (e.g., [4,10]).

Do these newly established synapses between the grafted neurons and the neurons of the recipient show normal functional characteristics? Several studies have contributed to progress towards a better understanding of the neurobiological substrates by which the grafted neurons may be functionally integrated into the host neural circuitry. Using a microdialysis technique, Nilsson et al. [41,42] have shown that basal forebrain grafts placed into the denervated hippocampus were able to release ACh. A major finding was the demonstration that these grafted cholinergic neurons were regulated by host afferents: in their grafted rats, both a sensory stimulation (stroking the fur and tail) and an electrical activation of the habenula were able to markedly increase the hippocampal ACh released by the grafted cells, an effect which was also observed in virtually intact rats but not in fimbria-fornix lesioned ones. More recently, Leanza et al. [33] showed this host-derived regulation of grafted neurons to involve catecholaminergic afferents from the host: in fimbria-fornix lesioned rats that sustained grafts rich in cholinergic neurons, a systemic apomorphine (2.0 mg/kg) or amphetamine (2.5 mg/kg) treatment increased ACh release to about 190% and 300% of baseline, respectively. All these findings strongly suggest that the activity of fetal cholinergic neurons implanted into the denervated hippocampus can be regulated by the host brain.

Beside the mechanisms involving afferences from other neurons, the release of a neurotransmitter by a given population of neurons is also subject to regulations involving presynaptic autoreceptors or heteroreceptors (e.g., [50]). Concerning the hippocampal cholinergic afferents in intact rats, it is known that evoked ACh release can be decreased subsequently to activation of either muscarinic autoreceptors or serotonergic heteroreceptors. In the rat, these heteroreceptors seem to belong to the 5-HT_{1B} subtype (e.g., [6,7,39]). A few data on other brain regions and species, some of them using in vivo techniques, suggest that 5-HT_{1A} receptors [3,25,55] and 5-HT_3 receptors [2,15,37] might also be involved in the modulation of ACh release, although contradictory findings have been reported for 5-HT_3 receptors [28].

Our present study was aimed at investigating whether the release of ACh by cholinergic neurons grafted into the denervated hippocampus of rats is regulated normally by muscarinic autoreceptors and serotonergic heteroreceptors. Sham-operated and lesion-only rats were used as controls. Using hippocampal slices preincubated with [^{3}H]choline and subsequently placed into superfusion chambers, the electrically-evoked release of [^{3}H]ACh was measured in the presence of muscarinic agonists (oxotremorine and, acting indirectly, physostigmine) and antagonists (atropine), or of drugs affecting 5-HT receptors, i.e. the non selective 5-HT_1/5-HT_2 antagonist methiothepin, the 5-HT_{1A} agonist 8-OH-DPAT, the 5-HT_3 agonist 2-methyl-serotonin, and the selective 5-HT_{1B} agonist CP 93129. Unfortunately, no selective 5-HT_{1B} antagonist is available at present [36]. The release measured without any drug application was used as the control condition. ACh release was also examined in the presence of a nicotinic antagonist, mecamylamine, as Potter and Nitta [45] have reported that after an AF64A-induced cholinergic hippocampal denervation, the inhibitory muscarinic receptors located on the spared cholinergic terminals were no longer operative, whereas facilitatory nicotinic receptors still worked. As additional controls for the effects of either the lesions or the grafts, we also assessed ChAT activity, noradrenaline (NA), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations, as well as the levels of [^{3}H]choline accumulated by hippocampal slices during the incubation.

2. Materials and methods

2.1. Materials

Chemicals and drugs were obtained from the following sources: [methyl-^{3}H]choline chloride (69–86 Ci/mmol) and [1-^{14}C]-Acetyl-coenzyme A (60 mCi/mmol) from Amersham Buchler, Braunschweig, Germany; atropine sulfate, hemicholinium-3, and oxotremorine sesquifumarate from Sigma, München, Germany; (±)-8-hydroxy-dipropylaminotetratin HBr (8-OH-DPAT), mecamylamine HCl, methiothepin mesylate, 2-methyl-5-hydroxytryptamine maleate (2-methyl-5-HT), and ritanserin from RBI (Biotrend), Köln, Germany. Finally, 3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-*b*]pyrid-5-one (CP 93129) was kindly donated by Dr. Schwegler, Pfizer GmbH, Karlsruhe, Germany.

2.2. Subjects

The study used 42 Long-Evans female rats obtained from R. Janvier (France). They were housed in Makrolon cages (59 × 38 × 20 cm) in groups of five or six. For lesion and transplantation surgeries, they were isolated in

smaller cages (42 × 26 × 15 cm) where they were kept for 3 days after surgery. Food and water were available ad libitum. The colony room was maintained on a 12:12 h light/dark cycle (lights on at 07.00) under controlled temperature (23 ± 1°C). All procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (council directive #87848, october 19, 1987, *Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animales*; permission #6212 to J.-C.C.)

2.3. Surgery

All surgery was performed under aseptic conditions, using equithesin anaesthesia (3 ml/kg, i.p.).

2.3.1. Lesion surgery

At 91 (±1) days of age, 29 rats received a bilateral electrolytic lesion of the infracallosal septo-hippocampal pathways (fimbria-fornix). This operation was performed by passing a rectified current of 1 mA for 40 s through an epoxylite coated stainless-steel electrode (0.15 mm in diameter) which was uninsulated at the tip (approx. 0.5 mm). The electrode was lowered into the brain at five sites according to the following coordinates: from Lambda [44], A = 5.2 mm, L = ±0.8 mm, V = −3.3 mm; A = 5.5 mm, L = 0.0 mm, V = −3.5 mm; A = 5.8 mm, L = ±1.8 mm, V = −3.8 mm. The incisor bar was placed 3.0 mm below the interaural line. The control group (Group 'Sham'; $n = 13$) consisted of rats which received scalp incision and removal of the bone overlying the dorsal parietal cortex.

2.3.2. Transplant surgery

Cells to be grafted were prepared from the brains of Long-Evans fetuses (CRL: 14 mm; E: 15 days). Two weeks after lesion surgery, a subgroup of lesioned rats (Group 'Graft'; $n = 13$) received bilateral intrahippocampal grafts of a cell suspension prepared from the region including the septal-diagonal band of Broca, a region rich in cholinergic neurons. The other subgroup of rats (Group 'Lesion'; $n = 16$) consisted of lesioned rats which did not receive grafts.

The grafts were performed as described by Cassel et al. [12]. Briefly, after extraction of the fetal brains, the ventral forebrain region containing the septal area was dissected out under a stereoscopic magnifier using sterile instruments and glassware. The dissection procedure of the fetal ventral forebrain was similar to that described by Björklund et al. [5]. The tissue fragments were collected in 0.6% glucose-saline, incubated for 20 min at 37°C in the same solution with 0.1% trypsin (Sigma, Grade II), washed three times with 5 ml of fresh glucose-saline and brought to a final volume of about 10 μl per septal tissue. These blocks were then dissociated using a fire-polished Pasteur pipette until obtention of a milky suspension. Injections (2 μl/site, 1 μl/min) of the resulting suspension were performed stereotaxically, through a Hamilton syringe, into each dorsal hippocampus at the following coordinates: from Lambda [44], A = 4.0 mm, L = ±1.5 mm, V = 3.1 mm; A = 3.0 mm, L = ±2.5 mm, V = 3.3 mm. The incisor bar was placed 3.0 mm below the interaural line. The syringe was left in situ for 2 min after each injection. Cell suspensions were used within a maximum of 3 h after preparation. The number of cells injected was counted in a haemocytometer (Thoma chamber, for details see [12]) and non-viable cells were identified with 0.05% Trypan blue. Counts of cells per μl were 52,500, with about 5% damaged, non-viable, cells.

2.4. Accumulation of [^{3}H]choline and evoked release of [^{3}H]ACh

2.4.1. Dissection of the hippocampus

Between 6.5 and 8 months post-grafting, the rats (Sham, $n = 11$; Lesion, $n = 13$; Grafted, $n = 11$) were decapitated and their brains were quickly removed. Each brain was sectioned transversally immediately before the dorsal edge of the hippocampus in order to separate a rostral and a caudal brain piece. The rostral piece was collected in 0.1 M phosphate-buffered 1.6% paraformaldehyde and kept at 4°C until it was sectioned for determination of the lesion extent (see below). From the caudal piece of the brain, both hippocampi were dissected free and cut such as to separate a portion including the two septal thirds of the hippocampus. The most dorsal thirds of the remaining ventral parts were homogenized and used for measurements of neurochemical parameters as described below.

2.4.2. Superfusion experiments

The dorsal portion was cut into 350 μm thick transversal slices. Starting at the septal pole, the first three slices were discarded and the next 14 slices from each hippocampus were transferred into a small Petri dish containing 2 ml Krebs-Henseleit (KH) buffer with [^{3}H]choline (0.1 μM) and incubated for 45 min at 37°C under carbogen. The KH solution had the following composition (in mM): NaCl, 118; KCl, 4.8; $CaCl_2$, 1.3; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4, 1.2; glucose, 11; ascorbic acid, 0.57; Na_2EDTA, 0.03; saturated with carbogen, pH adjusted to 7.4. After incubation, 24 slices were transferred into superfusion chambers (12 chambers per superfusion apparatus, 1 slice per chamber) and superfused with oxygenated KH buffer (37°C) at a rate of 0.6 ml/min. The superfusion medium was supplemented routinely with hemicholinium-3 (10 μM). In addition, either physostigmine (0.1 μM) was present throughout superfusion in experiments assessing the effects of cholinergic antagonists (atropine, mecamylamine), or ritanserin (1 μM) in experiments investigating the effects of serotonergic drugs (agonists and antagonists).

An electrical field stimulation (rectangular, 18 pulses, 3 Hz, 4 V/chamber, 24 mA) was applied 20 min after beginning of the superfusion. The collection of 4-min fractions was started 25 min later. [^{3}H]ACh release was induced by electrical field stimulations (360 rectangular pulses at 3 Hz, 4 V/chamber, 24 mA) after 57 min (S_1) and 89 min (S_2) of superfusion. In experiments using 3 stimulations, the third one (S_3) was applied after 121 min of superfusion. At the end of the experiment (after 141 min of superfusion), the radioactivity of superfusate samples and slices (dissolved in 500 μl Soluene 350, Packard, Frankfurt, Germany) was determined by liquid scintillation counting. Calculation of both the spontaneous outflow and the stimulation-evoked overflow of tritium was done as described previously [24].

2.4.3. Application of drugs

For each rat, the evoked release of [^{3}H]ACh was measured after application of different pharmacological substances (both cholinergic and serotonergic, as indicated in the legends to Figures) and their effects were compared to the appropriate control ratios (S_2/S_1 or S_3/S_1) without drugs. All drugs were added to the superfusion medium from 16 min before the corresponding electrical stimulation (S_2 or S_3) onwards.

2.4.4. Determination of specific [^{3}H]choline accumulation

From the slices which were not used for the release experiments, four slices per rat were processed in order to determine the proportion of *specific* [^{3}H]choline accumulation (i.e., the *hemicholinium-3-sensitive* fraction of [^{3}H]choline accumulated in the cholinergic terminals). The slices were incubated using the same conditions as for the superfusion experiments, but two of them were incubated in the presence of hemicholinium-3 (10 μM) in order to block the high affinity choline uptake into cholinergic nerve terminals. Following incubation, all four slices were superfused during 45 min (at a rate of 0.6 ml/min in the presence of hemicholinium-3, 10 μM) without any electrical field stimulation. Finally, they were solubilized in 500 μl 0.1 N NaOH before liquid scintillation counting. For each rat, the specific accumulation of [^{3}H]choline was expressed as a percentage calculated from the difference between the accumulation found in absence and that found in presence of hemicholinium-3. The average percentage found in each group was taken into consideration for correcting the [^{3}H] accumulation of the slices from the superfusion experiments with drugs and field-stimulations (Fig. 2).

2.5. Determination of ChAT activity and HPLC measurements

The most dorsal thirds of the remaining ventral parts of the hippocampi of the rats were used for determination of ChAT activity and HPLC measurements. They were collected in 2 ml of 0.32 M sucrose solution (in 2.5 mM HEPES, pH 7.4) and homogenized in a Potter/Elvehjem glass/teflon homogenizer (8 strokes at 500 rpm). From this crude homogenate, a 20 μl sample was used for determination of protein content, another 100 μl sample for measurements of ChAT activity and a further 700 μl aliquot was mixed with 700 μl 0.2 N $HClO_4$ (containing 250 mg Na_2SO_3 and 200 mg disodium EDTA per liter) and stored at −20°C for HPLC determinations.

ChAT activity was determined as described by Fonnum [23] with the modifications described previously [14]. The concentrations of noradrenaline, 5-HT and 5-HIAA were determined by HPLC with electrochemical detection as described previously [14], but using an electrochemical detector (ECD 460) and pump (model 510) from Waters. Protein content was assessed according to the method described by Lowry et al. [35].

2.6. Histological / histochemical verifications

Two sham-operated, three lesioned and two grafted rats not used in the aforementioned series of experiments were anaesthetized with an overdose of pentobarbital (100 mg/kg, i.p.) and transcardially perfused with 50 ml of 0.9% saline followed by 60 ml of 0.1 M phosphate-buffered 4% paraformaldehyde (4°C). After extraction, the brains were post-fixed for about 4 h and transferred into a 0.1 M phosphate-buffered 20% sucrose solution for 36–40 h. The brains were then quickly frozen and cut into 30 μm thick coronal sections using a cryostat (−20°C). From the posterior septum to the posterior region of the hippocampus, each fifth section was collected onto gelatine-coated slides. The sections were dried at room temperature for 36 hours and stained either with cresyl violet [49] or for acetylcholinesterase according to a method similar to that of Koelle [31]: ethopropazine (0.3 mM) was used to block non-specific cholinesterases and acetylthiocholine iodide (4 mM) was used as the substrate.

2.7. Statistical analysis

As regards [^{3}H]choline accumulation, the individual values were obtained from at least 22 slices per rat. The amount of ^{3}H release evoked by S1 was calculated for each rat using 11 slices per rat. In each other single superfusion experiment on hippocampal slices, the data from at least 3 slices per drug and concentration conditions were averaged for each rat. These average values (originating from at least five rats per surgical treatment) were analysed by an analysis of variance (ANOVA [56]) followed, where appropriate, by 2×2 comparisons based on the Duncan's multiple range test [51]. The ANOVA always considered the factor 'Group' and, where appropriate, the factors 'Drug' or 'Dose'.

3. Results

3.1. Extent of the lesion, distribution of AChE staining, ChAT activity and levels of noradrenaline, 5-HT and 5-HIAA

The lesions of the fimbria-fornix pathways were comparable to the lesions performed in previous experiments [26,27]. A typical example of such a lesion is shown in Fig. 1. Briefly, the lesions damaged the dorsal fornix as well as a major part of the fimbria. In only a few rats was the most ventral part of the lateral fimbria found to be almost intact. These lesions also slightly encroached onto the most dorsal portion of the lateral septum, the medial part of the overlying corpus callosum and onto the most anterior pole of the dorsal hippocampus. As illustrated in Fig. 1, the fimbria-fornix lesions induced a dramatic reduction of the hippocampal AChE-staining. The grafts consisted of well delineated, small cell aggregates which were densely AChE-positive and which had provided the denervated hippocampus with an organotypic AChE-positive reinnervation. They were located within the dentate gyrus (Fig. 1E) or close to region CA1 (Fig. 1F).

The lesion-induced cholinergic denervation of the hippocampus was also confirmed by the ChAT activity data. ANOVA of the data showed a significant overall Group effect ($F(2,29) = 9.12$, $P < 0.001$). In the homogenate prepared from more ventral parts of the hippocampus (a region not used in the superfusion experiments), the lesions had significantly reduced the ChAT activity to 43% ($\pm 5.1\%$) of the control values (sham-operated rats; $P < 0.05$). In the grafted rats, the average ChAT activity reached 53% ($\pm 4.8\%$) of the controls (Table 1); this reduction was also significant as compared to the sham-operated rats ($P < 0.05$). There was no significant difference between the lesioned and the grafted rats.

Lesion- and graft-induced effects were also studied by measuring tissue concentrations of 5-HT, its main metabolite 5-hydroxyindoleacetic acid (5-HIAA) and noradrenaline (NA). ANOVA of 5-HT concentrations showed a significant Group effect ($F(2,32) = 4.88$, $P < 0.05$) which was due to reduced 5-HT concentration in both lesioned

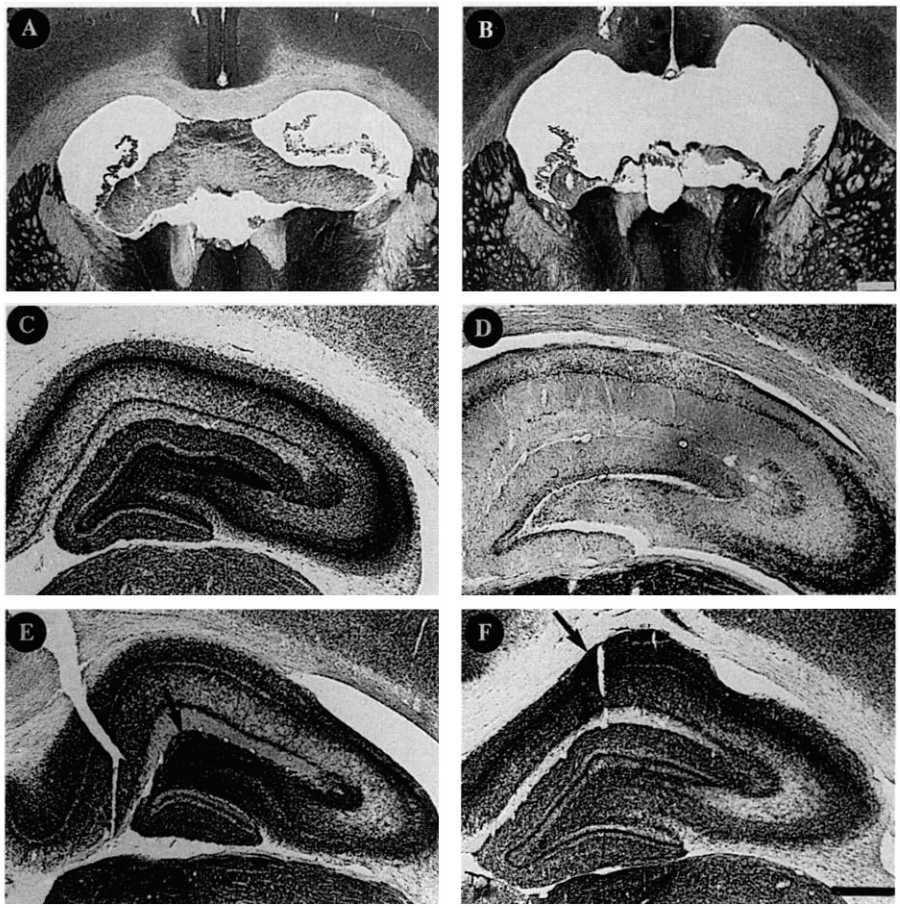

Fig. 1. B: photograph showing the extent of a representative fimbria-fornix lesion on a coronal section (stained for AChE) located about 1.3 mm posterior to Bregma [44]; compare to the intact fimbria-fornix in a sham-operated rat (A). AChE-positivity in a coronal section through the dorsal hippocampus of a sham-operated (C), a lesioned (D) and two grafted rats, one with a graft located within the dentate gyrus (E), the other one with a graft located partly within and partly above area CA1 (F). The arrows point onto the grafted tissue. White (A, B) and black (C–F) scale bars = 500 μm.

Table 1
Neurochemical measurements in a portion of the ventral hippocampus (next to the part used for superfusion experiments) of sham-operated, lesioned and grafted rats

Parameter measured	Sham	Lesioned	Grafted
ChAT activity (nmol/mg protein/min)	0.58 ± 0.07 (10)	0.25 ± 0.04 * (11)	0.31 ± 0.05 * (11)
Noradrenaline (ng/mg protein)	2.57 ± 0.26 (11)	3.59 ± 0.65 (13)	2.59 ± 0.37 (11)
5-HT (ng/mg protein)	2.25 ± 0.38 (11)	1.01 ± 0.20 * (13)	1.34 ± 0.29 * (11)
5-HIAA (ng/mg protein)	1.62 ± 0.31 (11)	1.02 ± 0.21 (13)	0.95 ± 0.22 (11)

Significant differences vs. sham-operated rats: * $P < 0.05$; number of rats given in parentheses.

and grafted rats as compared to the sham-operated ones ($P < 0.05$). The difference between the lesioned and the grafted rats was not significant. Due to high variabilities, the ANOVA failed to show a significant Group effect for either 5-HIAA or NA concentrations ($F(2,32) = 2.2$ and 1.0, respectively). Regarding 5-HIAA concentration, there was a tendency for this marker to be decreased in lesion-only rats as compared to sham-operated rats ($P < 0.10$). It is also noteworthy that tissue levels of hippocampal NA tended to be increased in lesioned rats ($P = 0.11$) as compared to the sham-operated ones, whilst the values found in grafted rats were almost identical to those of the virtually intact controls (Table 1).

3.2. [^{3}H]choline accumulation of hippocampal slices

Data are shown in Fig. 2. ANOVA of the 'total' (specific + unspecific) [^{3}H]choline accumulation values showed a significant overall Group effect ($F(2,32) = 7.2$, $P < 0.01$). This effect was due to a significant reduction of the values found in both the lesioned and the grafted rats as compared to the sham-operated ones ($P < 0.05$). In the grafted rats, the accumulation values only tended to be significantly higher than in the lesioned rats ($P = 0.10$). The proportion of *specific* [^{3}H]choline accumulation in the slices (i.e. of the hemicholinium-3 sensitive part, see Methods) was 72.7% (±2.5), 50.1% (±6.5) and 56.9 (±4.1) in the sham-operated, the lesioned and the grafted rats, respectively. ANOVA of the accumulation values corrected for specific [^{3}H]choline accumulation still showed a significant overall group effect ($F(2,32) = 30.3$, $P < 0.001$) which is interpreted exactly as for the absolute [^{3}H]choline accumulation data, excepted that the difference between grafted and lesion-only rats was now significant ($P < 0.05$).

3.3. Evoked overflow of tritium

The overflow of ^{3}H evoked by the first electrical stimulation (S_1), as expressed either in nCi or in percent of the

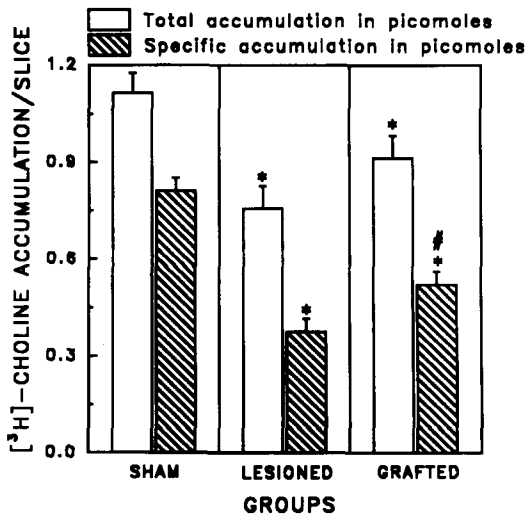

Fig. 2. Accumulation of [^{3}H]choline into hippocampal slices of sham-operated, lesioned and grafted rats: 'total' accumulation and values corrected for 'specificic' (hemicholinium-sensitive) [^{3}H]choline uptake (see Section 2). Mean values (±S.E.M., in picomoles [^{3}H]choline) of 11 sham-operated, 13 lesion-only and 11 grafted rats (data from at least 22 slices were averaged for each rat prior to statistical analysis). * Significant difference ($P < 0.05$) as compared to sham-operated rats; # significant difference ($P < 0.05$) as compared to lesion-only rats.

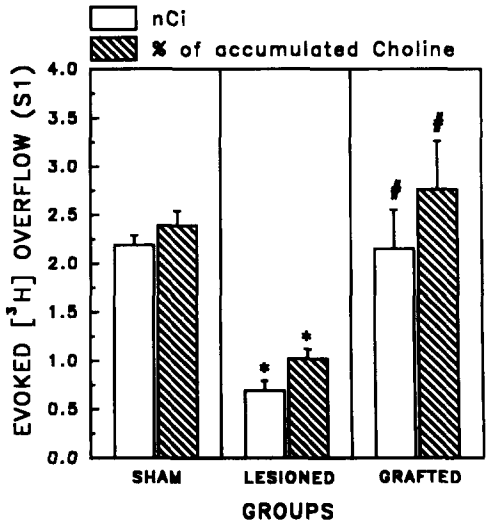

Fig. 3. ^{3}H overflow evoked by electrical field stimulation at S_1 in hippocampal slices (preincubated with [^{3}H]choline) of sham-operated, lesioned and grafted rats, shown as either 'absolute' (in nCi) or 'relative' values (in % of tissue $-^3$H). In all experiments only hemicholinium-3 (10 μM) was present throughout superfusion. Mean values (±S.E.M.) of 11 sham-operated, 13 lesion-only and 10 grafted rats (data from at least 11 slices were averaged for each rat prior to statistical analysis). * Significant difference ($P < 0.05$) as compared to sham-operated rats; # significant difference ($P < 0.05$) as compared to lesion-only rats.

^{3}H content of the slices before S_1 (in the presence of hemicholinium-3 alone throughout superfusion), is shown in Fig. 3. ANOVA of the absolute amount of ^{3}H overflow (in nCi) induced by this stimulation showed a significant Group effect ($F(2,31) = 25.2$, $P < 0.001$). This effect was due to a significant reduction of the overflow of ^{3}H in the lesioned as compared to the sham-operated rats (−65%, $P < 0.05$). The grafts contributed to compensate for this lesion-induced reduction: the average value in the grafted rats was significantly higher than in the lesioned rats ($P < 0.05$) and failed to differ significantly from that found in the sham-operated rats. ANOVA of the relative amounts (% of accumulated [^{3}H]choline) of evoked transmitter release also showed a significant Group effect ($F(2,31) = 18.3$, $P < 0.001$) which can be interpreted as above. As regards the relative amounts of transmitter release, it is noteworthy that half the grafted rats showed values (mean = 4.12 ± 0.49, $n = 5$) that largely exceeded the average value found in the sham-operated rats.

In the experiments investigating the effects of cholinergic antagonists (atropine, mecamylamine), the AChE inhibitor physostigmine (0.1 μM) was present throughout superfusion in addition to hemicholinium-3, whereas the effects of serotonergic drugs were determined in the additional presence of the 5-HT$_2$ receptor antagonist ritanserin (1 μM). The effects of these drugs on the electrically evoked [^{3}H]ACh release were assessed by comparing their influence on the overflow of ^{3}H at S_1 (Table 2). As evident from Table 2, ritanserin did not significantly change the evoked overflow of ^{3}H in any surgical treatment group ($F(1,57)$ and $F(2,57) < 1.0$, for the factor Drug and the Group × Drug interaction, respectively), whether considered in % of accumulated ^{3}H or in nCi. As regards the physostigmine effects, the ANOVA showed overall Group ($F(2,41) = 6.3$ (%) and 9.3 (nCi), $P < 0.01$) and Drug ($F(1,41) = 23.1$ (%) and 23.8 (nCi), $P < 0.001$) effects. The Drug effect was due to a significant physostigmine-induced reduction of the evoked [^{3}H]ACh release in the three groups ($P < 0.01$). The Group × Drug interactions tended to be significant ($F(2,41) = 2.7$ (%) and 2.6 (nCi), $P < 0.10$). This tendency was due to inhibitory effects of physostigmine that were significantly less pronounced in sham-operated (56 ± 4% inhibition) and lesioned rats (46 ± 6% inhibition) as compared to the grafted rats (70 ± 6% inhibition).

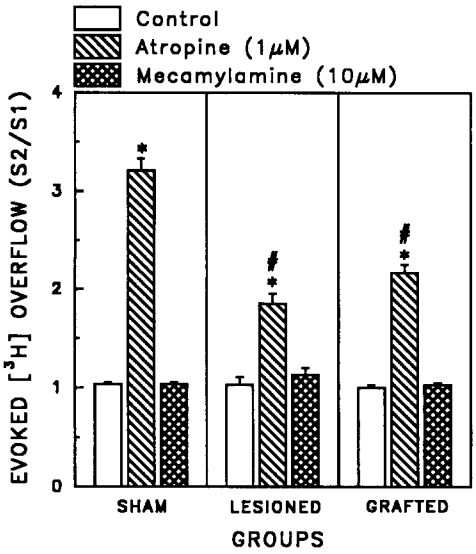

Fig. 4. Effects of atropine or mecamylamine on the electrically-evoked [^{3}H] overflow (expressed as the ratio S_2/S_1) in hippocampal slices (preincubated with [^{3}H]choline) of sham-operated, lesion-only and grafted rats. Atropine or mecamylamine were added to the medium from 16 min before S_2 onwards. Mean values (±S.E.M. in picomoles of [^{3}H]choline) of 5 sham-operated, 5 lesion-only and 5 grafted rats (data from at least 3 slices were averaged for each rat and each drug condition prior to statistical analysis). For the overflow evoked at S_1, see Table 2. * Significant difference ($P < 0.05$) as compared to the respective control value within each group; # significant difference ($P < 0.05$) as compared to the values found with the slices of sham-operated rats exposed to atropine.

3.4. Effects of atropine and mecamylamine

These experiments were performed in the presence of physostigmine (0.1 μM, see above) in order to increase the concentration of the endogenous agonist ACh. The data expressed as S_2/S_1 ratios are shown in Fig. 4. The Group (sham, lesion, graft) × Drug (control, atropine 1 μM,

Table 2
Electrically evoked overflow of ^{3}H at S_1 in the presence of various drugs present throughout superfusion

Drugs present throughout superfusion		Sham	Lesioned	Grafted
Hemicholinium-3	%	2.39 ± 0.15 (10)	1.02 ± 0.1 # (12)	2.76 ± 0.5 § (10)
	nCi	2.19 ± 0.1	0.69 ± 0.1 #	2.15 ± 0.4 §
Hemicholinium-3 + ritanserin	%	2.67 ± 0.2 (9)	1.15 ± 0.1 # (12)	2.34 ± 0.4 § (10)
	nCi	2.40 ± 0.07	0.72 ± 0.1 #	1.82 ± 0.3 §
Hemicholinium-3 + physostigmine	%	1.06 ± 0.06 * (5)	0.55 ± 0.03 *# (5)	0.82 ± 0.07 *#§ (5)
	nCi	0.98 ± 0.03 *	0.31 ± 0.06 *#	0.58 ± 0.01 *#§

Data are given in percentage of tissue − ^{3}H (%) and in nCi. Slices of the dorsal hippocampus from rats with different surgical treatments were preincubated with [^{3}H]choline and superfused (as described in Section 2) either in the presence of hemicholinium-3 (10 μM) alone, or in the additional presence of ritanserin (1 μM) or physostigmine (0.1 μM). Electrical stimulation was performed after 57 min of superfusion. Significant differences (in each surgical treatment group) as compared to the presence of hemicholinium-3 alone: * $P < 0.05$; as compared to sham-operated rats: # $P < 0.05$; as compared to lesioned rats: § $P < 0.05$; number of rats given in parentheses.

mecamylamine 10 μM) ANOVA showed significant effects of the factors Group ($F(2,12) = 43.8$, $P < 0.001$) and Drug ($F(2,24) = 354.1$, $P < 0.001$), as well as a significant Group × Drug interaction ($F(4,24) = 33.6$, $P < 0.001$). In all three groups, the application of atropine resulted in a significant increase of [^{3}H]ACh release ($P < 0.05$), whilst mecamylamine had no significant effect. The atropine-induced increase was significantly more pronounced in the sham-operated rats as compared to both the lesioned and the grafted ones ($P < 0.05$); the difference between the average values found in the two latter groups was not significant.

3.5. Effects of oxotremorine

The data were first expressed as either S_2/S_1 or S_3/S_1 ratios of the values found after application of different doses of oxotremorine and subsequently converted into a percentage of the appropriate control ratio (S_2/S_1 and S_3/S_1 without application of oxotremorine). These converted values were analysed using a Group × Dose (0.01–10 μM dose range) ANOVA (Fig. 5). The analysis showed a significant Group effect ($F(2,14) = 7.1$, $P < 0.01$), as well as a significant effect of the Dose ($F(3,42) = 541.8$, $P < 0.001$). There was no significant interaction between these two factors. Compared to the 100% control value in each group, oxotremorine was found to induce a significant reduction of the evoked [^{3}H]ACh release at the doses of 0.1, 1 and 10 μM ($P < 0.05$), the inhibition of the release being of about 20% at 0.1 μM and of slightly more than 80% at 10 μM. At the doses of 0.1, 1 and 10 μM, we found the evoked release to be significantly higher in lesioned rats as compared to the respective values found in sham-operated rats ($P < 0.05$). The grafted rats showed values intermediate between those of sham-operated and lesioned rats. The difference between grafted and lesioned rats was significant only at the dose of 0.1 μM ($P < 0.05$).

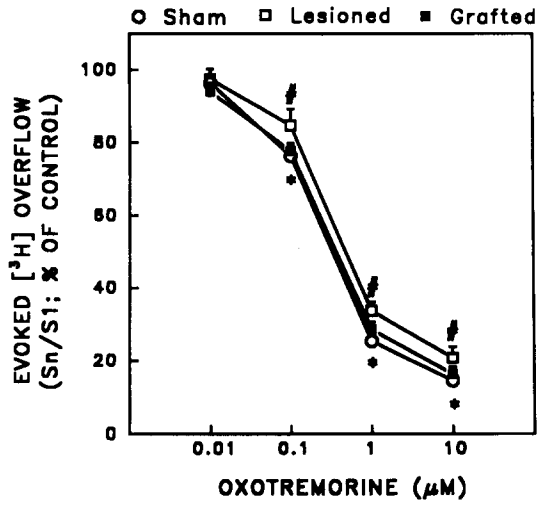

Fig. 5. Effects of oxotremorine on the evoked ^{3}H overflow in hippocampal slices (preincubated with [^{3}H]choline) of sham-operated, lesion-only and grafted rats. The effects of various concentrations of oxotremorine on the evoked ^{3}H overflow (S_2/S_1 or S_3/S_1 ratios) are expressed as a percentage of the respective control ratios (no oxotremorine, see text). The superfusion medium contained only hemicholinium-3 (10 μM) throughout superfusion; oxotremorine was added to the medium (in the concentrations indicated on the abscissa) from 16 min before S_2, or S_3, respectively, onwards. For the overflow evoked at S_1, see Table 2 and Fig. 3. Mean values (± S.E.M.) of 5 sham-operated, 7 lesion-only and 5 grafted rats (data from at least 4 slices were averaged for each rat and each drug condition prior to statistical analysis). * denotes significant ($P < 0.05$) oxotremorine-induced reduction in all three groups as compared to the respective control values (no oxotremorine); # denotes significant ($P < 0.05$) differences as compared to the values found with the slices of the sham-operated rats exposed to the same concentration of oxotremorine.

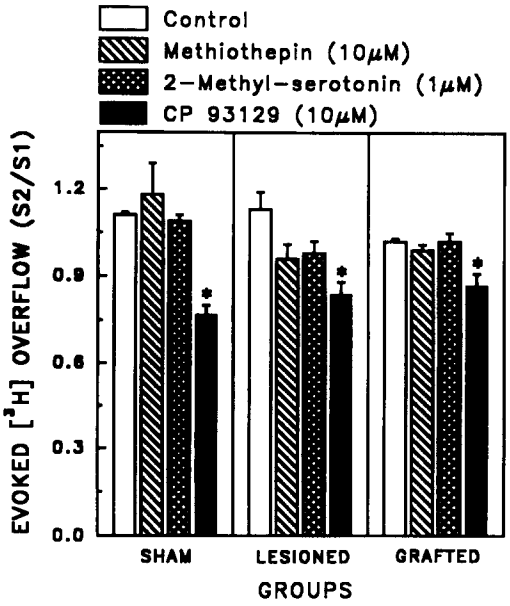

Fig. 6. Effects of serotonergic drugs on the evoked ^{3}H overflow (expressed as the ratio S_2/S_1) in hippocampal slices (preincubated with [^{3}H]choline) of sham-operated, lesion-only and grafted rats. In addition to hemicholinium (10 μM), the medium contained ritanserin (1 μM) throughout superfusion. Methiothepin, 2-methyl-serotonin or CP 93129 were added from 16 min before before S_2 onwards. For the overflows evoked at S_1, see Table 2. Mean values (± S.E.M.) of 4 sham-operated, 5 lesioned and 5 grafted rats (data from at least 3 slices were averaged for each rat and each drug condition prior to statistical analysis). * denotes significant differences ($P < 0.05$) as compared to the respective control value within each group.

3.6. Effects of 5-HT receptor agonists and antagonists

These experiments were performed in the presence of the 5-HT_2 antagonist ritanserin (1 μM) throughout superfusion. In the presence of the 5-HT_{1A} agonist 8-OH-DPAT at S_2 the evoked ^{3}H overflow from the slices was not significantly affected (results not illustrated; S_2/S_1 ratios were 1.06 ± 0.07 in sham rats, 1.16 ± 0.10 in lesioned rats and 1.0 ± 0.05 in grafted rats; control ratios were 1.11 ± 0.07, 0.98 ± 0.08 and 0.98 ± 0.10, respectively). Data (S_2/S_1 ratios) concerning the other serotonergic drugs are

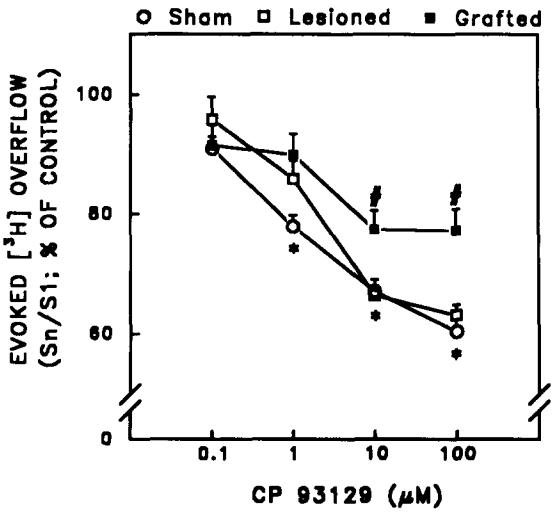

Fig. 7. Effects of CP 93128 on the evoked ^{3}H overflow in hippocampal slices (preincubated with [^{3}H]choline) of sham-operated, lesion-only and grafted rats. The effects of various concentrations of CP 93128 on the evoked ^{3}H overflow (S_2/S_1 or S_3/S_1 ratios) are expressed as a percentage of the respective control ratios (no CP 93128, see text). The superfusion medium contained hemicholinium-3 (10 μM) and ritanserin (1 μM) throughout superfusion; CP 93128 was added to the medium (in the concentrations indicated on the abscissa) from 16 min before S_2, or S_3, respectively, onwards. For the overflow evoked at S_1, see Table 2. Mean values (±S.E.M.) of 5 sham-operated, 7 lesion-only or 5 grafted rats (data from at least 4 slices were averaged for each rat and each drug condition prior to statistical analysis). * denotes significant ($P < 0.05$) CP 93128-induced reductions in all three groups as compared to the respective control values (no CP 93128); # denotes significant ($P < 0.05$) differences as compared to the values found with the slices of the sham-operated rats exposed to the same concentration of CP 93128.

depicted in Fig. 6. The Group × Drug ANOVA showed no significant Group effect, but both the Drug effect and the Group × Drug interaction were significant ($F(3,33) = 23.9$, $P < 0.001$; $F(6,33) = 3.8$, $P < 0.01$). Whilst methiothepin and 2-methyl-serotonin failed to produce a significant modification of [^{3}H]ACh release, CP 93129 significantly inhibited this release by 31% in sham-operated rats, 26% in lesioned rats and only 15% in grafted rats. The significant interaction can be explained by an inhibitory effect of CP 93129 which was more pronounced in both the sham-operated and the lesion-only rats than in the grafted ones. These inhibitory effects of CP 93129 on the evoked release of [^{3}H]ACh were also found when methiothepin (10 μM) was co-applied with the 5-HT_{1B} agonist, although this inhibition was now of similar amplitude in the three experimental groups (approx. −30%; data not shown). Methiothepin had no proper effect.

The experiment investigating the dose–response relationship between CP 93129 and [^{3}H]ACh release was performed according to a protocol similar to that in which several doses of oxotremorine were applied (see above). Data are presented in Fig. 7. The ANOVA showed significant Group ($F(2,14) = 6.1$, $P < 0.05$) and Dose ($F(3,42) = 87.2$, $P < 0.001$) effects, as well as a significant interaction between these two factors ($F(6,42) = 4.1$, $P < 0.01$). At the dose of 0.1 μM, the inhibition was significant only in sham-operated rats ($P < 0.05$). At higher doses, the inhibition was significant in all groups, although there was a marked between-group difference in the amplitude of the inhibition. In the grafted rats, the inhibition induced by 10 μM and 100 μM of CP 93129 was significantly less pronounced (−22,6% and −22.8%, respectively) than in either sham-operated (−33% and −39.6%, respectively) or lesioned rats (−33.6% and −36.9%, respectively).

4. Discussion

4.1. General observations on neurochemical parameters

In line with our previous findings [26,27], the present experiment confirms that electrolytic fimbria-fornix lesions induce, among other deficits, a severe cholinergic denervation of the hippocampus. This denervation was demonstrated by significant decreases in hippocampal AChE-positivity, ChAT activity, accumulation of [^{3}H]choline in hippocampal slices, and electrically-evoked ^{3}H overflow in hippocampal slices preincubated with [^{3}H]choline. Since electrically-evoked overflow of ^{3}H from brain slices preincubated with [^{3}H]choline has been shown many times (e.g., [24]) to reflect action potential-induced, exocytotic release of ACh, only the expression 'evoked ACh (or [^{3}H]ACh) release' will be used hereafter.

As regards the graft-induced effects, we also confirm that grafts rich in cholinergic neurons are able to attenuate the cholinergic deficit due to the lesions. The grafts actually provided the hippocampus with a new AChE-positive innervation, increased (but did not normalize) the accumulation of [^{3}H]choline in hippocampal slices, and normalized the evoked release of [^{3}H]ACh in absence of any drug application. If one excepts the failure of our grafts to significantly increase the hippocampal ChAT activity, all these findings are in good agreement with many other reports [13,14,18,22,27,29,30,52].

A methodological explanation to the fact that the grafts failed to significantly affect the ChAT activity in the denervated hippocampus can be proposed. Actually, in a previous experiment by Jeltsch et al. [27], we found electrolytic fimbria-fornix lesions to reduce ChAT activity by about 70% in the dorsal part of the hippocampus and by only about 40% in the ventral part. Grafts rich in cholinergic neurons placed into the dorsal hippocampus were found to significantly increase ChAT activity in the dorsal hippocampus, but they failed to exert significant effects in its ventral part, probably because the graft-derived fibers failed to grow into this distal region. In our present experiment, the activity of ChAT was assessed on homogenates prepared from the hippocampal tissue pieces next to those used in the superfusion experiments (part of

ventral region). Therefore, the detection of significant graft-induced effects could simply not be expected in the material that we used to prepare the homogenates because of a too ventral neuroanatomical origin of the slices that were homogenized. These samples, nevertheless, allowed to detect lesion-induced effects, a finding which is consistent with our previous report [27].

Also, HPLC data on tissue levels of 5-HT, 5-HIAA and noradrenaline are in agreement with our previous observations on more ventral parts of the hippocampal formation [14,26]: serotonin concentration was significantly diminished in both lesion-only and grafted rats, whereas noradrenaline levels tended to be increased in the former but were close to normal in the latter group. This confirms our previous reports [9,13] showing that septal grafts do not affect serotonergic parameters and that graft-derived cholinergic reinnervation of the hippocampus may prevent a lasting increase of hippocampal noradrenaline concentration induced by septohippocampal lesions. Although we did not find the lesion-induced increase of noradrenaline concentration to be significant in our present experiment, this increase was found to be significant in two former experiments [9,14] in which the lesions of the fimbria-fornix were larger (aspiration of the infracallosal and supracallosal pathways) than in the present study (electrolysis of only the infracallosal pathways). The denervation-induced increase in hippocampal noradrenaline levels may be due to a sprouting response of (peripheral) sympathetic neurons innervating cerebral microvessels [16].

4.2. Modulation of ACh release

In our three experimental groups, we have determined the efficacy of two types of mechanisms that may exert a presynaptic modulation of ACh release in the hippocampus. One of these mechanisms involves an inhibitory muscarinic autoreceptor which – in the rat hippocampus – probably belongs to the M2 type [46,47], although McKinney et al. [40] have provided some evidence that septohippocampal terminals in the rat might possess receptors of the M4 type. In this regard, it is noteworthy that Waelbroeck et al. [53] assigned 22% of the *N*-[^{3}H]methylscopolamine binding sites in the rat hippocampus to the M4 subtype and 12% to the M2 type, respectively. Our experimental design did not allow to address the question of which subtype of receptors is responsible for the observed effects on evoked [^{3}H]ACh release.

The other presynaptic inhibitory mechanism involves a serotonergic heteroreceptor which is presumably of the 5-HT_{1B} type, a possibility consistent with our findings showing that neither 8-OH-DPAT (a selective 5-HT_{1A} agonist), nor 2-methyl-serotonin (a selective 5-HT_3 agonist) produced any significant effect on evoked [^{3}H]ACh release, whereas CP 93129 (a selective 5-HT_{1B} agonist (see [36]) did significantly and dose-dependently attenuate this release. Our experiments were performed in the presence of the 5-HT_2 receptor antagonist ritanserin, since it has been shown [2] that the 5-HT_3 agonist 2-methyl-serotonin inhibited ACh release in cortical slices only in its presence. Interestingly, the nonselective 5-HT_1/5-HT_2 receptor antagonist methiothepin was without any facilitatory effect even in sham-operated rats. This observation indicates that under the present experimental conditions, an inhibitory tone of endogenously released 5-HT was not detectable. Recently, several studies using the in vivo microdialysis technique suggested [3,25,55] that hippocampal ACh release might also involve modulatory 5-HT_{1A} receptors. Since 8-OH-DPAT showed no effect on ACh release from brain slices in vitro (present report and [39]), it must be concluded that 5-HT_{1A} receptors modulating ACh release in vivo are located on neurons of polysynaptic loops rather than on cholinergic axon terminals themselves. In conclusion, all observations of the present study are in line with previous reports upon pharmacological properties of 5-HT receptors on cholinergic terminals in the intact rat hippocampus (e.g., [6,7,38,39]).

In addition to previous experiments that have demonstrated the activity of cholinergic neurons grafted into the denervated hippocampus to be regulated by host afferents (e.g., [33,41,42]), our results show that the release capabilities of such neurons may also be regulated by mechanisms involving muscarinic autoreceptors and serotonergic heteroreceptors of the 5-HT_{1B} type. A major contribution of the present experiment is the demonstration that both the muscarinic autoreceptors and the serotonergic heteroreceptors show a sensitivity which may depend upon the surgical treatment to which the rats have been submitted.

4.3. Sensitivity of muscarinic autoreceptors

Before discussing this point, it is important to recall that ACh release experiments realized on hippocampal slices of sham-operated (or intact) rats differ in one important aspect from those performed on slices prepared from lesion-only or grafted rats. Indeed, in material from intact rats, the targets of the superfused cholinergic drugs mainly consist of the receptors located on the cholinergic nerve terminals (i.e. *presynaptic* receptors). Conversely, in slices from grafted rats, *somatodendritic* receptors located on the grafted cholinergic neurons are additional targets that may be involved in the regulation of cholinergic activity and ACh release.

As regards the modulation of ACh release via muscarinic autoreceptors, several findings of the present study deserve discussion. First, in hippocampal slices of grafted rats, the absolute amount of evoked ACh release (values in nCi) did not differ from that found in sham-operated rats; in half of the grafted rats, the relative amount (in % of tissue tritium) of evoked ACh release was even largely higher than in the sham group, an observation suggesting that, in this subpopulation of grafted rats, a higher amount of ACh per stimulus and per nerve terminal was released.

That not all grafted rats have presented such an increase might be due to some fluctuations in the quality of the transplant's integration. Unfortunately, our experimental protocol did not allow us to perform histological verifications of this quality in the rats used for the superfusion experiments as all slices prepared from the hippocampal tissue pieces were necessary because of statistical constraints. Second, the effect of the AChE inhibitor physostigmine was more pronounced in slices from grafted rats as compared to both lesion-only and sham-operated rats. Since physostigmine (as an indirect cholinomimetic drug) increases the concentration of ACh in the vicinity of the axon terminals ('biophase concentration of ACh'), i.e. near the inhibitory muscarinic autoreceptors, this finding again supports the notion of an increased ACh release per stimulus. Third, the facilitatory effect of atropine (in the presence of physostigmine) on the evoked ACh release was significantly weaker in slices from grafted rats as compared to those from sham-operated rats. Although the latter finding could also be explained by the increased biophase concentration of ACh (which competes with atropine at the receptor), an alternative possibility would be that the muscarinic autoreceptor in the hippocampus of grafted and lesioned rats underwent a downregulation, leading to a decreased sensitivity towards atropine and thereby to an increased amount of evoked ACh release per stimulus and nerve terminal.

However, in the experiments using application of oxotremorine (in the absence of physostigmine), such a downregulation was found to be significant only in the lesioned rats. In these rats, the oxotremorine-induced decrease of [^{3}H]ACh release was actually less pronounced than in the intact control rats. The values found in the grafted rats were intermediate between those found in the groups of sham-operated and lesioned rats but differed significantly from neither. At present, the reason for which we could not demonstrate a diminished sensitivity of the muscarinic autoreceptor towards oxotremorine in grafted rats is unclear. One possible explanation is that the exogenous agonist oxotremorine may have sites of action located on grafted neurons which do not exist on the cholinergic terminals of sham-operated rats. In grafted rats, these sites might be somatodendritic muscarinic receptors (see above).

In the lesioned rats, the downregulation of muscarinic autoreceptors can be considered as an adaptive change occurring in the terminals of the cholinergic neurons spared by the lesion procedure. This adaptive change is probably based on an attenuation of the autoinhibitory processes at the level of the cholinergic terminals that survived the lesion procedure, thereby contributing to increased cholinergic function in the preserved neurons. Such a downregulation of the autoreceptors might be a direct consequence of an increased turnover in the undamaged cholinergic neurons. This possibility is in agreement with the report of Lapchak et al. [32] who showed that after a partial lesion of the fimbria-fornix pathways, the undamaged cholinergic neurons of the septohippocampal system upregulate their capacity to synthesize and store ACh. That these neurons might also increase their capacity to release greater amounts of stored ACh is indirectly suggested by the presence of downregulated muscarinic autoreceptors on the cholinergic terminals, as suggested by our present results.

Recently, Potter and Nitta [45] reported that after AF64A-induced lesions of the cholinergic hippocampal afferents, the remaining cholinergic terminals completely lost their ability to regulate the release of ACh via muscarinic receptors, whilst the regulatory mechanisms involving facilitatory nicotinic receptors were not only conserved, but even exacerbated. In our lesioned rats, we did not observe such an exacerbation, a failure that might probably be explained by the differences in the respective lesion methods (electrolysis of the fimbria and the fornix v.s i.c.v. injections of AF64A), stimulation characteristics (360 pulses, 3 Hz, 4 V vs. 240 pulses, 2 Hz, 30 V), incubation procedures (slices not stimulated during incubation vs. slices stimulated) or drug application protocols (physostigmine-containing buffer throughout the superfusion experiment vs. no physostigmine throughout), and other technical differences. Further experiments are certainly required to address this particular question in more detail.

Regarding previous experiments, one could expect the grafts rich in cholinergic neurons to attenuate or even to counterbalance some of the neurochemical or pharmacological alterations induced by the lesions (e.g., [13,14,18,22,27,29,30,52]). In our present experiment, such expectations were verified on parameters such as [^{3}H]choline accumulation in the slices or [^{3}H]ACh release in absence of any drug application (see above). It is noteworthy, however, that in some respects, a muscarinic downregulation similar to that found in the lesioned rats was also observed on the terminals of the grafted neurons, at least under the condition of atropine application. This finding may appear as somewhat surprising regarding previous reports showing that the lesion-induced alterations of muscarinic sensitivity in the hippocampus may be counterbalanced by grafts rich in cholinergic neurons (e.g., [18,29,54]). However, as discussed in these reports, the lesion- and graft-induced effects did probably involve muscarinic receptors located at a postsynaptic level. At the presynaptic level, our findings may suggest that the grafted cholinergic neurons have developed some abnormal (or failed to develop normal) pharmacological characteristics as regards the muscarinic autoinhibitory control mechanisms of ACh release. The modification of the pharmacological properties found at the terminals of the grafted cholinergic neurons does probably reflect a functional facilitation in the grafted cholinergic neurons, in particular as regards the release of ACh.

Beside the findings reported by Dawson et al. [18] or by Joyce et al. [29], our present results suggest that some modifications in the sensitivity of muscarinic receptors

may also occur at the presynaptic level of cholinergic neurons which have survived the lesion or which have been grafted into the hippocampus.

4.4. Sensitivity of serotonergic heteroreceptors

As was the case for the muscarinic autoreceptors, we also found the sensitivity of the 5-HT_{1B} heteroreceptors to depend upon the surgical treatment of the rats. Actually, the evoked release of [^{3}H]ACh in presence of 10 or 100 μM of CP 93129 was reduced to a level which was significantly lower in both the sham-operated and the lesioned rats (approx. −33% and −38%, respectively) as compared to the grafted ones (approx. −23%). This difference between the grafted rats and the rats from the two other groups suggests that the 5-HT_{1B} receptors located on the terminals of the grafted cholinergic neurons have been downregulated or have failed to express normal functional characteristics as regards the serotonin-mediated mechanisms involved in the presynaptic regulation of ACh release. In addition to the muscarinic dowregulation that was found in our grafted neurons, the abnormality in the sensitivity of 5-HT_{1B} receptors might be considered as another type of adaptive change which, at the terminals of the grafted cholinergic neurons, might be considered as beneficial as it may contribute to increase the graft-derived cholinergic function in the hippocampus.

A theoretical explanation for this 5-HT_{1B} downregulation in the grafted cholinergic neurons would be that the grafts contained serotonergic neurons that tonically released 5-HT. However, a previous experiment has demonstrated that intrahippocampal septal tissue grafts prepared according to the method used in the present experiment did not alter the lesion-induced effects on serotonergic markers in the hippocampus [27], an observation suggesting that these grafts were devoid of serotonergic neurons. In the present experiment, our HPLC data confirmed these observations. In previous experiments [26,27], we repeatedly found lesions identical to those performed in the present experiment to severely reduce some serotonergic markers in the dorsal hippocampus (i.e., synaptosomal serotonin uptake, serotonin and 5-hydroxyindoleacetic acid concentrations) and to increase the ratio of 5-hydroxyindoleacetic acid and serotonin concentrations. The latter modification indicates that the serotonin turnover has been upregulated in the neurons spared by the lesions, an upregulation by which serotonergic fibers tend to compensate for the reduced serotonergic innervation of the hippocampus (e.g., [1,14,17]). Theoretically, it is possible to consider that such an upregulation of the turnover might account for a downregulated sensitivity of the serotonergic receptors in the close vicinity of the spared serotonergic terminals. Nevertheless, if such an explanation would be appropriate to account for our present findings in the grafted cholinergic neurons, a 5-HT_{1B} downregulation should also have been observed in the undamaged cholinergic neurons of the lesion-only rats. As this was not the case, one may be tempted to propose another (speculative) explanation based on consideration of abnormal maturation of some pharmacological characteristics of the cholinergic terminals originating in the grafted tissue.

Actually, the cholinergic neurons which were grafted at a stage of complete immaturity may have failed to express a completely normal phenotypic set of pharmacological characteristics over the 6.5–8 months long postgrafting survival time. In other words, it is possible that in a serotonin-depleted cellular environment, thus in absence of a normal level of serotonergic inputs, the grafted cholinergic neurons failed to express a 5-HT_{1B} sensitivity comparable to that found in the hippocampus of intact rats. This highly speculative hypothesis would certainly deserve further experimentation. For instance, it might be interesting to compare the sensitivity of 5-HT_{1B} receptors when cholinergic neurons are implanted with a procedure identical to that used in our present experiment (single grafts), or when these neurons are implanted in combination with a preparation rich in serotonergic neurons (co-grafts of two different neurochemical populations of cells), the latter being able to provide the denervated hippocampus with a new serotonergic innervation (e.g., [27,41]).

4.5. Conclusions

The results of our present experiment confirm that grafts of basal forebrain cholinergic neurons provide the denervated hippocampus with new cholinergic terminals that are, in some respects, functional as they are able to release ACh. These results also demonstrate that the grafted cholinergic neurons express some of the pharmacological characteristics found on cholinergic terminals in the hippocampus of normal rats. In particular, the terminals of these neurons possess functional muscarinic autoreceptors and 5-HT_{1B} heteroreceptors, which both contribute to reduce the release of ACh when they are activated. However, the functional properties of these receptors were not completely normal, as both showed a lower sensitivity than that found in normal rats. Both types of downregulations, however, may contribute to increase cholinergic function in the grafted tissue by reducing the inhibitory tonus that ACh and serotonin exert at the level of the cholinergic terminals.

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