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Neurotransmitter release and its presynaptic modulation in the rat hippocampus after selective damage to cholinergic or/and serotonergic afferents

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ABSTRACT: Male Long–Evans rats sustained injections of 5,7-dihydroxytryptamine (5,7-DHT) into the fimbria-fornix and the cingular bundle or/and intraseptal injections of 192 IgG-saporin to induce serotonergic or/and cholinergic hippocampal denervations; Sham-operated rats served as controls. Four to ten weeks after lesioning, we measured (i) the electrically evoked release of acetylcholine ([^{3}H]ACh), noradrenaline ([^{3}H]NA) and serotonin ([^{3}H]5-HT) in hippocampal slices in the presence of drugs acting on auto- or heteroreceptors, (ii) the nicotine-evoked release of NA and (iii) the choline acetyltransferase (ChAT) activity and the concentration of monoamines in homogenates. Saporin lesions reduced the accumulation of [^{3}H]choline, the release of [^{3}H]ACh and the ChAT activity, but increased the concentration of NA and facilitated the release of [^{3}H]NA evoked by nicotine. 5,7-DHT lesions reduced the accumulation and the release of [^{3}H]5-HT, the concentration of 5-HT, and also facilitated the release of [^{3}H]NA evoked by nicotine. Accumulation and electrically evoked release of [^{3}H]NA were not altered by either lesion. The combination of both toxins resulted in an addition of their particular effects. The 5-HT$_{1B}$ receptor agonist, CP 93,129, and the muscarinic agonist, oxotremorine, reduced the release of [^{3}H]ACh in control and 5,7-DHT-lesioned rats; in rats injected with saporin, their effects could not be measured reliably. CP 93,129 and the α_2-adrenoceptor agonist, UK 14,304, reduced the release of [^{3}H]5-HT in all groups by about 65%. In conclusion: (i) selective neurotoxins can be combined to enable controlled and selective damage of hippocampal transmitter systems; (ii) 5-HT exerts an inhibitory influence on the nicotine-evoked release of NA, but partial serotonergic lesions do not influence the release of ACh at a presynaptic level and (iii) presynaptic modulatory mechanisms involving auto- and heteroreceptors may be conserved on fibres spared by the lesions.
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KEY WORDS: 192 IgG-saporin, 5,7-Dihydroxytryptamine, Acetylcholine, CP 93,129, Noradrenaline, Oxotremorine, Serotonin, UK 14,304.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease that has become a topic of increasing interest during the last decades. In order to learn more about both the processes taking place in a degenerating brain and their relations to behavioural alterations, attempts have been made to develop animal models simulating aspects of AD from a neuroanatomical, neurochemical or/and behavioural point of view (e.g. [35,39]). Many such models were based on the idea that the cognitive impairments found in AD (principally recent memory impairments in the early stages of the disease) are due to the loss of cholinergic fibres in structures of the basal forebrain innervating the cortex and the hippocampus; this idea led to the so-called "cholinergic hypothesis of age-related cognitive dysfunctions" [3,4,14,16]. Although alterations of cholinergic systems are recognised to play a prominent role in dysfunctions related to AD, or even at a less dramatic level to ageing, it is noteworthy that other neurotransmitter systems, such as the serotonergic, noradrenergic or GABAergic systems, are also involved in cognitive functions [12,17].

Using neurotoxins, it is possible to selectively destroy one or more of these systems in order to study their functional implications. It is even possible to combine some neurotoxic lesions to investigate the role of interactions between transmitter systems. For instance, the cholinergic neurons of the basal forebrain can be selectively damaged by 192 IgG-saporin [48] and the serotonergic neurons of the mesencephalic raphé by 5,7-dihydroxytryptamine (5,7-DHT; [44]). Recently, using an approach relying upon the combined administration of 192 IgG-saporin and 5,7-DHT, Lehmann and coworkers [29,30] have confirmed that the cooperation between cholinergic and serotonergic mechanisms took part in cognitive functions such as spatial working memory (see also [5,9–11]). One of these studies [29] focused on the hippocampus, where, as is the case in any transmitter system of the brain or the periphery, the release of acetylcholine (ACh) and serotonin (5-HT) is regulated both by extrinsic and intrinsic (local) neuropharma-

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cological mechanisms. The intrinsic mechanisms modulating the neurotransmitter release involve auto- and heteroreceptors located on axon terminals [45]. In the hippocampus, the modulation of this release involves, e.g., M_2 autoreceptors and 5-HT_{1B} heteroreceptors on cholinergic terminals, 5-HT_{1B} autoreceptors and α_2 heteroreceptors on the serotonergic ones, and α_2 autoreceptors and nicotine heteroreceptors (nAChRs) on the noradrenergic ones [45]. Among these few receptors, only the nAChR on the noradrenergic terminals is excitatory. Knowing whether and how these transmitter systems and their receptors work after lesions or in models of degenerative disorders may be of importance as they may also be potential targets for drug therapies aimed at enhancing or blocking neurotransmission.

In a series of previous studies, we have started to investigate such modulatory mechanisms in the denervated hippocampus. To that end, we have used nonselective hippocampal denervation models (aspirative or electrolytic lesions of the fimbria-fornix pathways) combined or not with intrahippocampal grafting of fetal neurons (e.g. [13,26,28,43]). It was found that all auto- and heteroreceptors investigated (e.g., M_2, 5-HT_{1B}, α_2, nAChR) showed more or less close-to-normal functional characteristics (though some of them indicated weak up- or downregulations), whether on the spared terminals in lesion-only rats or on the terminals of grafted neurons. There is, however, a potential problem with such unselective lesions, namely that the release and the modulatory mechanisms investigated on the terminals of a particular neurotransmitter system may also be influenced by damage to other systems, rather than only by changes at the level of presynaptic receptors.

Therefore, the present study aimed at furthering these investigations using neurotransmitter-specific lesions. Using slices from the dorsal two thirds of the hippocampus selectively deprived of cholinergic or/and serotonergic afferents, we characterised the electrically evoked release of ACh, noradrenaline (NA) and 5-HT, as well as their modulation by auto- and heteroreceptors. The ACh- and nicotine-evoked release of NA (*via* nAChRs) was also investigated. The lesions were produced by injections of 192 IgG-saporin into the septal region and by injections of 5,7-DHT into the fimbria-fornix/cingular bundle pathways. Biochemical markers such as the ChAT activity or 5-HT, 5-hydroxyindolacetic acid (5-HIAA) and NA concentrations were also measured in a ventral portion of the hippocampus in order to estimate the extent of the denervations.

MATERIALS AND METHODS

Chemicals and Drugs

Chemicals and drugs were obtained from the following sources: [1-^{14}C]acetyl coenzyme A (52 mCi/mmol) and [methyl-^{3}H]choline chloride (82 Ci/mmol) from Amersham-Pharmacia, Freiburg, Germany; hemicholinium-3 from Chemcon, Freiburg, Germany; 5-[1,2-^{3}H(*N*)]hydroxytryptamine creatinine sulphate ([^{3}H]5-HT, 21.8 Ci/mmol) and [^{3}H]norepinephrine (55 Ci/mmol) from NEN Life Sciences, Frankfurt, Germany; 6-nitro-2-(1-piperazinyl)-quinoline (6-nitroquipazine) from RBI, Biotrend, Köln, Germany; acetylcholine chloride, acetyl coenzyme A, desipramine hydrochloride, hexamethonium chloride, nicotine hydrogen tartrate, oxotremorine sesquifumarate, physostigmine hemisulphate and tetrodotoxin from Sigma Aldrich Chemie GmbH, Steinheim, Germany; mecamylamine hydrochloride from Merck, Sharpe and Dohme; 3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrollo[3,2-b]pyrid-5-one (CP 93,129) was a gift from Dr. Pagani, Pfizer Inc., Groton, USA, and 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline tartarate (UK 14,304) a gift from Pfizer, Sandwich, Kent, UK.

Subjects

All procedures involving animals and their care were conducted in conformity with the international 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. and 6714-bis to H. J.; O. L. under the responsibility of J.-C. C.) and international (NIH publication, no. 80-23, revised 1996) laws and policies.

The study used 36 (21 for electrically evoked transmitter release and 15 for NA release experiments induced by nAChR stimulation, respectively) male Long–Evans rats aged of 3 months and weighing about 320 g at the time of surgery. They were housed individually in transparent Makrolon cages (42 cm × 26 cm × 15 cm) under a 12 h/12 h dark/light cycle (lights on at 0700 h), with *ad lib.* access to food and water. The colony and testing rooms were under controlled temperature (21°C).

Surgery

All surgical procedures were performed under aseptic conditions, using sodium pentobarbital anaesthesia (65 mg/kg, i.p.; Sanofi, France). All rats sustained two surgical operations conducted in two steps. In the first step, rats were subjected to an injection into the fimbria-fornix/cingular bundle (FiFx/CB) of either 8 μg of 5,7-DHT (in 0.64 μl of saline containing 20 mg/ml ascorbic acid; Sigma, St. Louis, USA) or its vehicle (0.64 μl). Twenty minutes before anaesthesia, all rats were injected with desipramine (25 mg/kg, i.p., in saline) to protect the noradrenergic system from the 5,7-DHT (e.g. [8]).

In the second step, 1 week later, rats were subjected to an injection into the medial septum/vertical limb of the diagonal band of broca of either 0.4 μg of 192 IgG-saporin (in 0.4 μl of phosphate-buffered saline (PBS), Chemicon, Temecula, CA, USA) or its vehicle (0.4 μl PBS).

All injections were performed stereotaxically through a 1 μl-Hamilton syringe according to coordinates of the Paxinos and Watson brain atlas [40]. For injection of 5,7-DHT or its vehicle, coordinates were (in mm from Lambda): AP $= +5.7$, ML $= \pm 0.9$, DV $= -4.0$ for the fimbria-fornix (0.2 μl/site); AP $= +5.7$, ML $= \pm 0.4$, DV $= -2.2$ for the cingular bundle (0.12 μl/site). The incisor bar was set at 3.0 mm below the interaural line. For injection of 192 IgG-saporin or its vehicle, coordinates were (in mm from Bregma): AP $= +0.6$, ML $= \pm 0.2$, DV $= -7.2$ for the vertical limb of the diagonal band of broca (vBDB; 0.1 μl/site) and DV $= -6.5$ for the median septum (MS; 0.1 μl/site). The incisor bar was set at the level of the interaural line. On each site, the needle was left *in situ* after each injection for 6 min before being retracted slowly.

In summary, rats received injections of NaCl into the FiFx/CB followed by 0.4 μg of 192 IgG-saporin into the MS/vBDB (saporin lesion, SAPO, $n = 9$), injections of 8 μg of 5,7-DHT followed by PBS (5,7-DHT lesion, DHT, $n = 9$) or both injections of neurotoxins (combined lesions, SAPO + DHT, $n = 10$). Sham-operated rats received both injections of vehicle (SHAM, $n = 8$).

Dissection of the Hippocampus and Neurochemical Determinations

Four to ten weeks after lesion surgery (and 13–14 weeks for the experiments on nAChR-evoked release of NA), the rats were sacrificed by decapitation, their brains quickly removed, both hippocampi dissected free and the ventral third of each hippocampus divided in two. One half of the ventral third was homogenised in 1 ml 0.32 M sucrose (in 2.5 mM HEPES, pH 7.4) in a Potter Elvehjem glass/teflon homogeniser (8 strokes at 500 rpm). From this crude homogenate, a 20-μl sample was diluted with 180 μl 0.1 N NaOH and used for measurement of protein according to Lowry

et al. [32]. A 100-μl sample of the homogenate was stored at −80°C until determination of ChAT activity according to Fonnum [19]. A 300-μl aliquot was mixed with 300 μl of 0.2 N $HClO_4$ (containing 250 mg Na_2SO_3 and 200 mg Na_2EDTA/l) and stored at −80°C until determination of NA, 5-HT and 5-HIAA by HPLC with electrochemical detection. Following thawing, the latter samples were centrifuged (10 min at 17,000 × *g*; 4°C) and the supernatants filtered using Millex-GV4 (0.22 μm; Millipore GmbH, Eschborn, Germany) filters. Twenty microlitres of the filtrated supernatants were injected onto a reversed phase HPLC column (Luna 5 μm C8; 250 mm × 4.6 mm column; Phenomenex, Aschaffenburg, Germany) and separation was performed at 30°C at a flow of 1 ml/min (pump model 480, Gynkotek, München, Germany). The mobile phase had the following composition (per litre): 3.9 g sodium acetate, 4.0 g citric acid (monohydrate), 114 mg 1-octanesulfonic acid (sodium salt), 35.7 mg Na_2EDTA, 138.1 ml methanol and 7.15 ml ethanol (pH adjusted to 4.2 with acetic acid). The amounts of the monoamines and 5-HIAA were determined using the electrochemical detector INTRO (Antec, Leyden, The Netherlands) set at 800 mV (at 30°C) and a standard calibration curve. The detection limits were 3 pg for NA, 6 pg for 5-HIAA and 7.5 pg for 5-HT.

Accumulation and Electrically Evoked Release of [^{3}H]Choline, [^{3}H]5-HT or [^{3}H]NA

The two dorsal thirds were cut in 350 μm slices using a McIlwain tissue chopper. The slices were distributed to three Petri dishes containing 2 ml Krebs–Henseleit (KH) buffer with either [^{3}H]choline (0.1 μM for ACh release experiments), [^{3}H]5-HT (0.1 μM for 5-HT release experiments) or [^{3}H]NA (0.1 μM for NA release experiments), 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, 10; ascorbic acid, 0.6; 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; for ACh release experiments, 9 chambers), 6-nitroquipazine (1 μM; for 5-HT release experiments, 9 chambers) or with desipramine (1 μM; for NA release, 6 chambers). Fractions (4 min) to be measured were collected from 45 min of superfusion onwards.

The release of [^{3}H]ACh, [^{3}H]5-HT or [^{3}H]NA, respectively, was induced by two electrical field stimulations (360 rectangular pulses at 3 Hz, 2 ms, 4 V/chamber, 26–28 mA) after 53 min (S_1), and after 81 min (S_2) of superfusion. Drugs to be tested were added to the superfusion medium of some chambers from 12 min before the second stimulation onwards. At the end of the experiment (after 101 min of superfusion) the radioactivity of superfusate samples and slices (dissolved in 250 μl Soluene 350, Packard, Frankfurt, Germany) was determined by liquid scintillation counting. The "fractional rate of tritium outflow" (in percent of tissue tritium per minute) was calculated as: (pmoles tritium outflow per minute) × 100/4 × (pmoles tritium in the slice at the start of the corresponding 4-min period). The "basal tritium outflow" in the fraction preceding S_1 (i.e., from 49 to 53 min of superfusion) is given either in *absolute* terms (nCi [^{3}H]outflow/4 min) or in *relative* terms ("fractional rate of tritium outflow per 4 min"). The "stimulation-evoked overflow of tritium" was calculated by subtraction of the basal outflow and is shown either in *absolute* terms ("nCi" [^{3}H]overflow) or in *relative* terms (in percent of the tritium content of the slice at the onset of the respective stimulation period). Effects of drugs added before S_2 were determined as the ratio of the overflow evoked by the corresponding stimulation period (S_2/S_1) and compared to the appropriate control ratio (no drug addition before S_2).

nAChR Stimulation-Evoked Release of [^{3}H]NA

Hippocampal slices (eight slices from each rat) preincubated with [^{3}H]NA as above were superfused at a rate of 0.6 ml/min in chambers (160 μl total volume) with oxygenated KH medium (37°C) devoid of any reuptake inhibitor. Fractions (4 min) to be measured were collected from 45 min of superfusion onwards. During superfusion these slices were stimulated once (S_1) after 61 min of superfusion by addition of either nicotine (100 μM), or ACh (100 μM, in the presence of physostigmine (1 μM)) for 2 min to the superfusion medium (rapid changes from the agonist-free to the agonist-containing and back to the agonist-free KH buffer was assured by 4-way valves). Determinations of radioactivity and calculations of the baseline and the nicotine-evoked [^{3}H]overflow were done as above.

Statistical Analysis

As regards [^{3}H] accumulation in the slices ([^{3}H]choline, [^{3}H]5-HT or [^{3}H]NA) and the average amount of [^{3}H]overflow evoked by S_1, mean values ± SEM were obtained from the pooled single values of all the rats subjected to the same surgical treatment (from each rat, up to 53 slices). Concerning the effects of drugs on electrically evoked [^{3}H]overflow, the corresponding S_2/S_1 ratios of slices from rats with the same surgical and experimental treatment were pooled. All data were analysed by parametric analysis of variance (ANOVA) or by the Kruskal–Wallis test (nonparametric ANOVA). Where appropriate, they were followed by 2 × 2 comparisons based on the Newmann–Keuls test (parametric) or on the multiple comparisons test of Dunn (nonparametric). *T*-tests were also used where appropriate [49].

TABLE 1

ChAT ACTIVITY (nmoles/mg PROTEIN/min) AND CONCENTRATION OF MONOAMINES (ng/mg PROTEIN) IN HOMOGENATES PREPARED FROM THE MOST VENTRAL PORTION OF THE HIPPOCAMPUS OF SHAM ($n = 8$), SAPO ($n = 9$), DHT ($n = 9$) AND SAPO + DHT ($n = 10$) RATS

	Groups			
Variable	SHAM	SAPO	DHT	SAPO + DHT
ChAT activity	0.63 ± 0.04	0.07 ± 0.02 (−89%)*	0.69 ± 0.05 (+10%)	0.04 ± 0.01 (−94%)*
Concentration of 5-HT	4.69 ± 0.44	5.44 ± 0.42 (+16%)	2.08 ± 0.21 (−56%)*	2.68 ± 0.35 (−43%)*
Concentration of 5-HIAA	5.40 ± 0.66	4.81 ± 0.59 (−11%)	2.36 ± 0.33 (−56%)*	3.19 ± 0.26 (−41%)*
Concentration of NA	5.99 ± 0.52	8.69 ± 0.73 (+45%)*	5.73 ± 0.34 (−4%)	7.70 ± 0.83 (+29%)

Values are the mean ± SEM. The percent variation compared to controls is indicated in parentheses.
* Significantly different from SHAM rats, $p < 0.05$.

RESULTS

Neurochemical Determinations

In order to quantify the changes in the density of cholinergic, serotonergic and noradrenergic innervation caused by selective cholinergic and serotonergic lesions, several neurochemical markers were measured in the ventral part of the hippocampus (not used for slice preparations). These data are shown in Table 1. ANOVA of the ChAT activity showed a significant Group effect [$F(3,32) = 110.58$, $p < 0.001$], which was due to a significant reduction of activity in SAPO (−89%) and SAPO + DHT (−94%) rats as compared to their Sham-operated counterpart, but also to DHT rats ($p < 0.001$, for all comparisons). ANOVA of the concentrations of 5-HT and 5-HIAA also showed a significant Group effect [$F(3,32) = 19.64$ and 8.75, respectively, $p < 0.001$], which was due to a significant reduction of the concentration of both markers in DHT (−56% in each) and SAPO + DHT rats (−43 and −41%) as compared to the Sham-operated rats ($p < 0.01$ for all comparisons). The 5-HIAA/5-HT ratios did not differ significantly among groups [$F(3,32) = 1.87$; not shown]. Finally, ANOVA of the NA concentration also yielded a significant Group effect, which was due to a significantly higher concentration of NA in SAPO rats as compared to SHAM (+45%) or to DHT rats ($p < 0.05$ in both cases). In SAPO + DHT rats, the concentration of NA was also higher than in Sham-operated rats (+29%), but failed to reach significance ($p = 0.08$).

Accumulation of [^{3}H]Choline, [^{3}H]5-HT or [^{3}H]NA by Hippocampal Slices

Figure 1 shows the accumulation of [^{3}H] in hippocampal slices preincubated with [^{3}H]choline, [^{3}H]5-HT and [^{3}H]NA. ANOVA of [^{3}H]choline accumulation values (Fig. 1A) showed a significant Group effect [$F(3,184) = 29.0$, $p < 0.001$], which was due to an accumulation of [^{3}H]choline that was significantly reduced in rats from groups SAPO (−25%) and SAPO + DHT (−40%), as compared to group SHAM or DHT ($p < 0.001$). Also, in SAPO + DHT rats, the [^{3}H]choline accumulation was significantly lower than in SAPO rats ($p < 0.01$).

ANOVA of the [^{3}H]5-HT accumulation values (Fig. 1B) also showed a significant Group effect [$F(3,182) = 61.28$, $p < 0.001$], which was due to a significant reduction of the accumulation of [^{3}H]5-HT in DHT (−56%) and SAPO + DHT rats (−42%), as compared to SHAM or SAPO rats ($p < 0.001$ in all cases). Also, in SAPO + DHT rats, the [^{3}H]5-HT accumulation was significantly higher than in DHT rats ($p < 0.05$).

Concerning the accumulation of [^{3}H]NA (Fig. 1C), ANOVA showed no significant Group effect [$F(3,121) = 1.13$].

Absolute Basal Outflow of [^{3}H]

Following preincubation with either [^{3}H]choline, [^{3}H]5-HT or [^{3}H]NA, hippocampal slices were superfused continuously with medium containing the choline reuptake inhibitor, hemicholinium-3, the 5-HT reuptake inhibitor, 6-nitroquipazine, or the NA reuptake inhibitor, desipramine, for ACh release experiments, 5-HT release experiments or NA release experiments, respectively. During superfusion, release of tritiated transmitters from dorsal hippocampal tissue was triggered by electrical field stimulation. In Fig. 2, examples of the fractional rates of basal and evoked [^{3}H]outflow (relative in percent of tritium accumulation) from hippocampal slices (means ± SEM) of the four groups of rats (SHAM, 5,7-DHT, SAPO and SAPO + 5,7-DHT) are shown. Although both basal and evoked outflow of tritium represent a mixture of tritiated compounds, for convenience, we will use the expression "outflow" (or "evoked overflow") of [^{3}H]ACh, [^{3}H]5-HT or [^{3}H]NA, hereafter, for slices incubated with [^{3}H]choline, [^{3}H]5-HT or [^{3}H]NA, respectively.

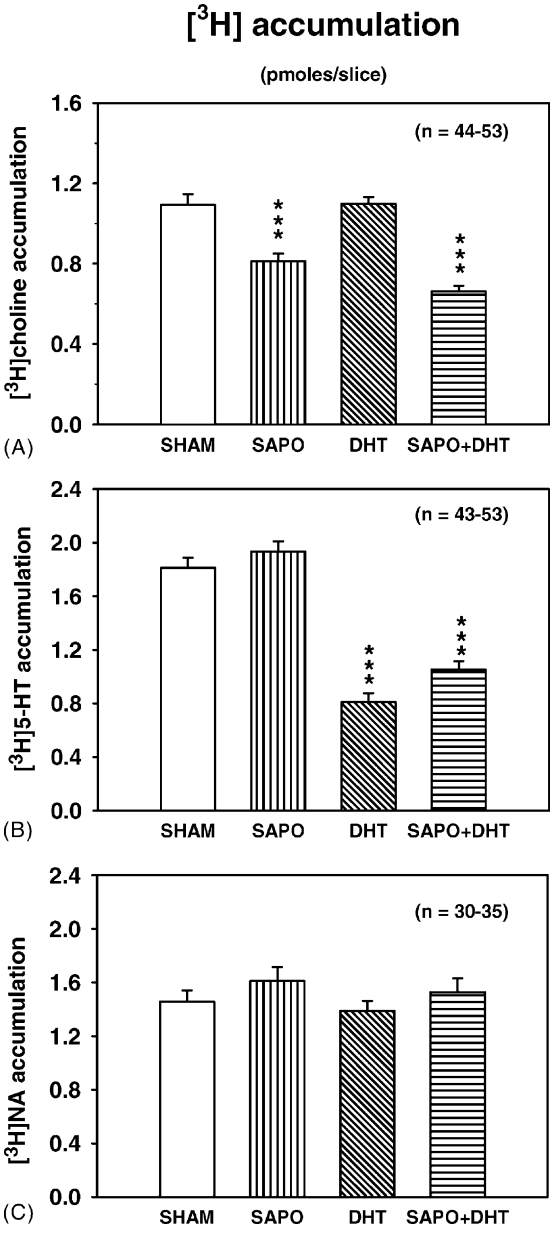

FIG. 1. Accumulation of [^{3}H] in hippocampal slices preincubated with [^{3}H]choline (A), [^{3}H]5-HT (B) or [^{3}H]NA (C). Data are the mean ± SEM [^{3}H] accumulation expressed in pmoles/slice. Number of rats per group: five to six; the minimal and maximal numbers of slices per column are indicated in parentheses. *** Significantly different from SHAM, $p < 0.05$ (see Section "RESULTS" for other differences).

The effects of the lesions on the basal outflow of [^{3}H]choline, [^{3}H]5-HT or [^{3}H]NA from hippocampal slices were investigated by comparing the fractional rates of [^{3}H]outflow (expressed either as percent of tissue [^{3}H] or in nCi) in the fraction immediately preceding S_1 (see Fig. 2). Hereafter, absolute outflow refers to values in nCi and relative outflow to values in percent of tissue [^{3}H].

Figure 3A shows that the cholinergic lesion had a clear-cut effect on the *absolute* basal outflow of [^{3}H]ACh, an observa-

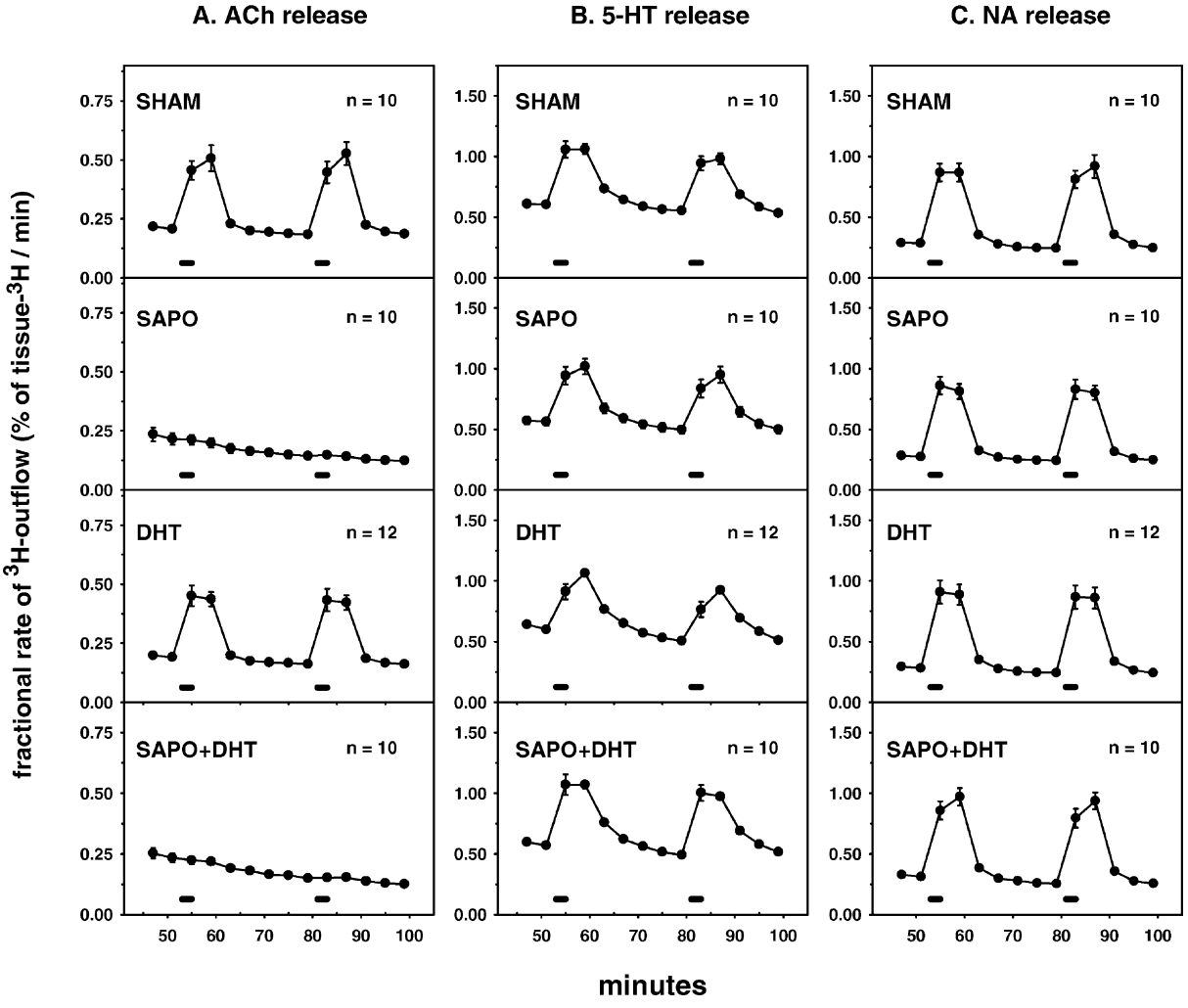

FIG. 2. Time course of [^{3}H]outflow (±SEM) from slices of the dorsal rat hippocampus preincubated with [^{3}H]choline (A), [^{3}H]5-HT (B) or [^{3}H]NA (C). The hippocampal slices were prepared from Sham-operated rats (SHAM), rats subjected to 192 IgG-saporin lesions (SAPO), to 5,7-DHT lesions (DHT) or to a combination of both lesions (SAPO + DHT). After incubation, the slices were superfused continuously in the presence of the appropriate reuptake blocker (see Section "MATERIALS AND METHODS") and, at two occasions (after 53 and 81 min of superfusion) exposed to an electrical field stimulation (S_1 and S_2; see horizontal bars). The [^{3}H]outflow per minute is expressed as a percentage of tissue [^{3}H] at the start of the corresponding fraction. The number of slices considered per group of rats is shown in the upper corner of each figure.

tion confirmed by an ANOVA that revealed a significant Group effect [$F(3,184) = 25.91$, $p < 0.001$]. Two by two comparisons showed that this effect was due to a basal outflow that was significantly lower in SAPO (−31%) and SAPO + DHT (−44%) rats, as compared to Sham-operated rats, but also to DHT rats ($p < 0.001$). Furthermore the outflow was significantly lower in the SAPO + DHT as compared to the SAPO rats ($p < 0.05$).

Serotonergic lesions reduced the *absolute* basal outflow of [^{3}H]5-HT as shown by a significant Group effect [$F(3,181) = 59.62$, $p < 0.001$]. This effect was due to significantly lower basal outflow values in the DHT and SAPO + DHT rats ($p < 0.001$) as compared to SHAM and SAPO rats. Moreover, the *absolute* basal outflow in DHT rats was significantly lower than in SAPO + DHT rats ($p < 0.05$).

There was no significant Group effect on the *absolute* basal outflow of [^{3}H]NA [$F(3,121) < 1.0$; Fig. 3C].

Relative Basal Outflow of [^{3}H]

As to the *relative* basal outflow of [^{3}H], ANOVA of the [^{3}H]choline values (Fig. 3A) showed a significant Group effect [$F(3,184) = 3.32$, $p < 0.05$] due to values that were significantly lower in DHT as compared to SAPO + DHT rats.

Also the ANOVA of the *relative* basal outflow of [^{3}H]5-HT revealed a significant Group effect [$F(3,182) = 7.73$, $p < 0.001$; Fig. 3B]. It was due to an outflow that was significantly lower in all three lesion groups (DHT: −7%; SAPO: −13%; SAPO + DHT: −11%) as compared to group SHAM ($p < 0.05$, at least).

The *absolute* basal outflow of [^{3}H]NA (Fig. 3C) was not altered by the lesions [$F(3,121) = 1.25$].

Absolute Evoked Overflow of [^{3}H]ACh, [^{3}H]5-HT and [^{3}H]NA

From Fig. 4A, it becomes clear that the cholinergic lesions had a dramatic effect on the *absolute* evoked overflow of [^{3}H]

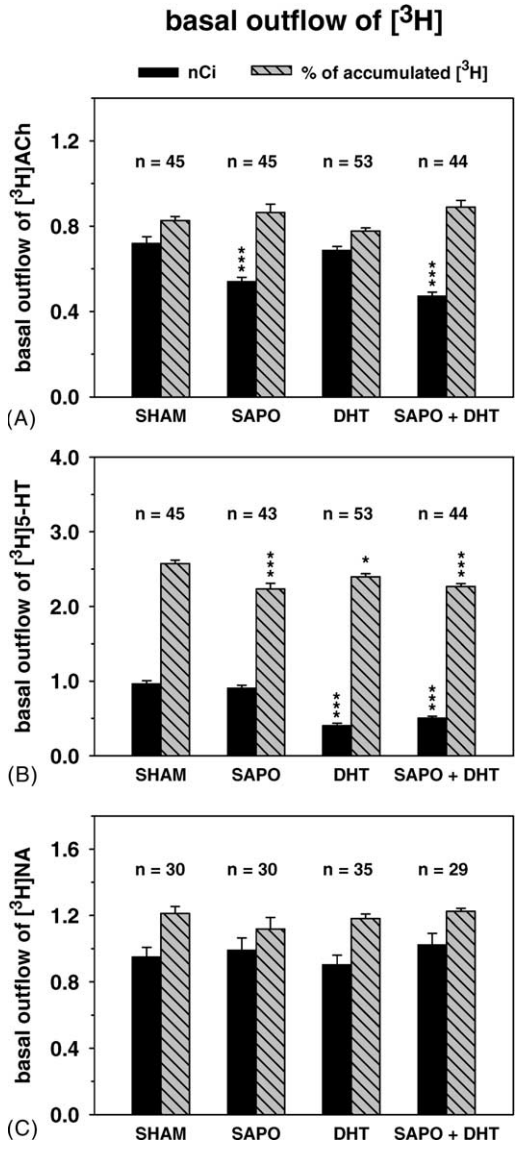

FIG. 3. Basal outflow of [^{3}H] in hippocampal slices preincubated with [^{3}H]choline (A), [^{3}H]5-HT (B) or [^{3}H]NA (C). Data are the mean ± SEM. The outflow of [^{3}H] is indicated as an absolute (in nCi; solid bars) or a relative (in percent of tissue [^{3}H]; hatched bars) accumulation. Data were from Sham-operated rats (SHAM), rats subjected to 192 IgG-saporin lesions (SAPO), to 5,7-DHT lesions (DHT) or to a combination of both lesions (SAPO + DHT). Number of rats per group: five to six; the number of slices considered per group is indicated above the bars. * Significantly different from SHAM, $p < 0.05$, *** $p < 0.001$ (see Section "RESULTS" for other differences).

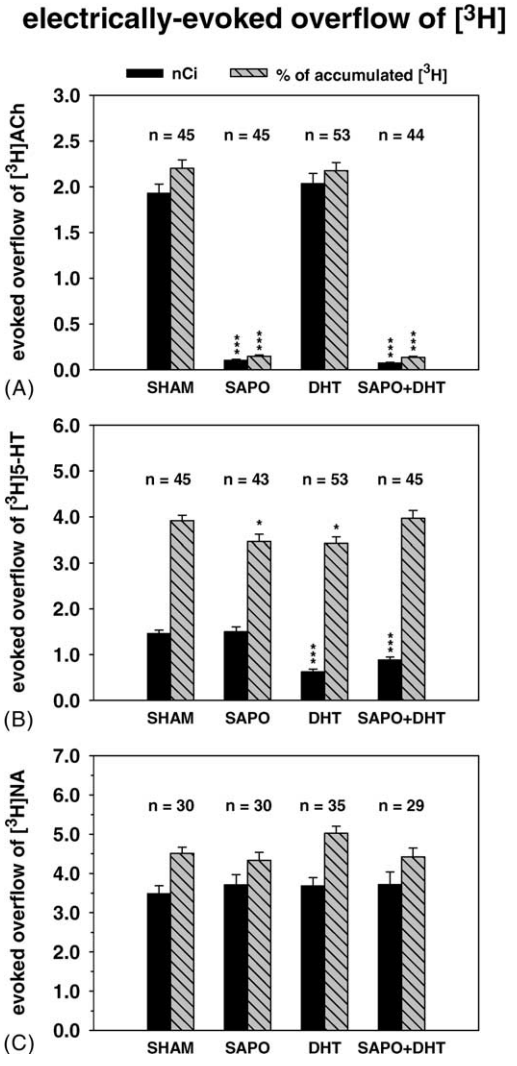

FIG. 4. Electrically evoked outflow of [^{3}H] in hippocampal slices preincubated with [^{3}H]choline (A), [^{3}H]5-HT (B) or [^{3}H]NA (C). Data are the mean ± SEM. The outflow of [^{3}H] is indicated as an absolute (in nCi; solid bars) or a relative (in percent of tissue [^{3}H]; hatched bars) accumulation. Data were from Sham-operated rats (SHAM), rats subjected to 192 IgG-saporin lesions (SAPO), to 5,7-DHT lesions (DHT) or to a combination of both lesions (SAPO + DHT). Number of rats per group: five to six; the number of slices considered per group is indicated above the bars. * Significantly different from SHAM, $p < 0.05$, *** $p < 0.001$ (see Section "RESULTS" for further differences).

(S_1 in nCi). This was confirmed by an ANOVA, which showed a significant Group effect [$F(3,183) = 180.12$, $p < 0.001$] due to values that were significantly lower in SAPO (−95%) and SAPO + DHT (−94%) rats, as compared to SHAM, but also to DHT rats ($p < 0.001$, in each case).

ANOVA of the *absolute* evoked overflow of [^{3}H]5-HT (Fig. 4B, solid bars) also showed a significant Group effect [$F(3,182) = 31.55$, $p < 0.001$]. This effect reflected a release that was significantly reduced in DHT rats as compared to SHAM (−57%), SAPO or SAPO + DHT rats, and in SAPO + DHT rats (−40%) as compared to either of the three other groups ($p < 0.001$).

Concerning the evoked *absolute* release of [^{3}H]NA (Fig. 4C), ANOVA showed no significant Group effect [$F(3,121) < 1.0$].

Relative Evoked Overflow of [^{3}H]ACh, [^{3}H]5-HT and [^{3}H]NA

It is evident, that the cholinergic lesions also had a dramatic effect on the *relative* amount of [^{3}H] released (S_1 in percent of tissue [^{3}H]), as shown in Fig. 4A. This was confirmed statistically, as the ANOVA showed a significant Group effect [$F(3,183) = 307.20$, $p < 0.001$], which was due to a significant reduction of the relative release of [^{3}H]ACh in SAPO (−93%) and SAPO + DHT

(−94%) rats, as compared to the SHAM, but also to the DHT rats ($p < 0.001$).

ANOVA of the relative amount of [^{3}H]5-HT released (Fig. 4B) also showed a significant Group effect [$F(3,182) = 3.98$, $p < 0.001$], which was due to the fact that the release in DHT rats was significantly lower than in SHAM rats (−13%). Also in SAPO rats was the release significantly lower than in SHAM (−12%) or SAPO + DHT rats ($p < 0.05$). In SAPO + DHT rats, the release was significantly higher than in DHT rats ($p < 0.05$).

Finally, as concerns the relative release of [^{3}H]NA (Fig. 4C), ANOVA also showed a significant Group effect [$F(3,121) = 2.77$, $p < 0.05$], which was due to a release that was significantly higher in DHT rats as compared to SAPO rats ($p < 0.05$).

Effects of Auto- and Heteroreceptor Agonists on Evoked ACh Release

The data were first expressed as S_2/S_1 ratios of the values found after application of a drug and subsequently converted into a percentage of the appropriate control ratio (S_2/S_1 without application of the drug). Modulation of the evoked [^{3}H]ACh release *via* 5-HT$_{1B}$ heteroreceptors and muscarinic autoreceptors was studied by measuring the effects on the electrically evoked overflow of the 5-HT$_{1B}$ agonist CP 93,129 and the muscarinic agonist oxotremorine (1 μM, each).

Addition of CP 93,129 to the superfusion medium before S_2 induced an inhibition of the evoked [^{3}H]overflow in groups SHAM (to 72.8 ± 2.5% of control) and DHT (to 71.2 ± 1.3% of control), an observation supported by a *t*-test showing a highly significant difference between CP 93,129 and control values ($p < 0.001$). In the two other groups, modulation of the evoked overflow by CP 93,129 could not be measured reliably as there was almost no release in response to the electrical stimulation (see Fig. 2). Nevertheless, it might be of some interest to indicate that the average release (in percent of control) amounted 97.9 ± 15.8 in SAPO rats, and 78.3 ± 13.1 in SAPO + DHT rats. From the few slices (at most six) that showed a weak release, we found that CP 93,129 reduced the [^{3}H]overflow by about 50% in SAPO and SAPO + DHT rats.

Addition of oxotremorine before S_2 also induced a clear-cut inhibition of the evoked overflow of [^{3}H]ACh in SHAM (to 34.1 ± 2.3% of control) and DHT (to 30.9 ± 1.5% of control) rats. In both groups, this reduction, as shown by a *t*-test, was also highly significant ($p < 0.001$). Once again, in both other groups, modulation of the evoked overflow could not be measured reliably, the evoked overflow being much too weak (see Fig. 2). Just in case, the average release (in percent of control) amounted 90.4 ± 18.4 in SAPO rats, and 51.2 ± 12.3 in SAPO + DHT rats. From the few slices that showed a weak release, we found that oxotremorine reduced the [^{3}H]overflow by about 60% in SAPO and SAPO + DHT rats.

Effects of Auto- and Heteroreceptor Agonists on Evoked 5-HT Release

Figure 5 shows the effects of the 5-HT$_{1B}$ agonist, CP 93,129, and the α_2 adrenoceptor agonist, UK 14,304 (1 μM, each), added before S_2 on the evoked 5-HT release from hippocampal slices of the four groups of rats. As evident from Fig. 5A, in all four groups, the 5-HT release was reduced by CP 93,129. This was confirmed by an ANOVA, which showed a significant Drug effect [$F(1,116) = 1220.0$, $p < 0.001$], but neither a significant overall Group effect, nor a significant interaction between the two factors [$F(3,116) < 1$, in both cases].

UK 14,304 also reduced the evoked overflow of [^{3}H]5-HT in all four groups (Fig. 5B) and, as demonstrated by an ANOVA, this reduction was significant: there was a significant Drug effect [$F(1,116) = 1215.3$, $p < 0.001$], but neither an overall Group effect nor an interaction between these two factors. It should be noted, however, that two by two comparisons of the effects of UK 13,304 revealed a significant stronger inhibition in the group DHT as compared to the group SHAM ($p < 0.05$). A similar comparison of the groups SAPO + DHT and SHAM did not reach significance level, although a tendency was also seen ($p = 0.08$).

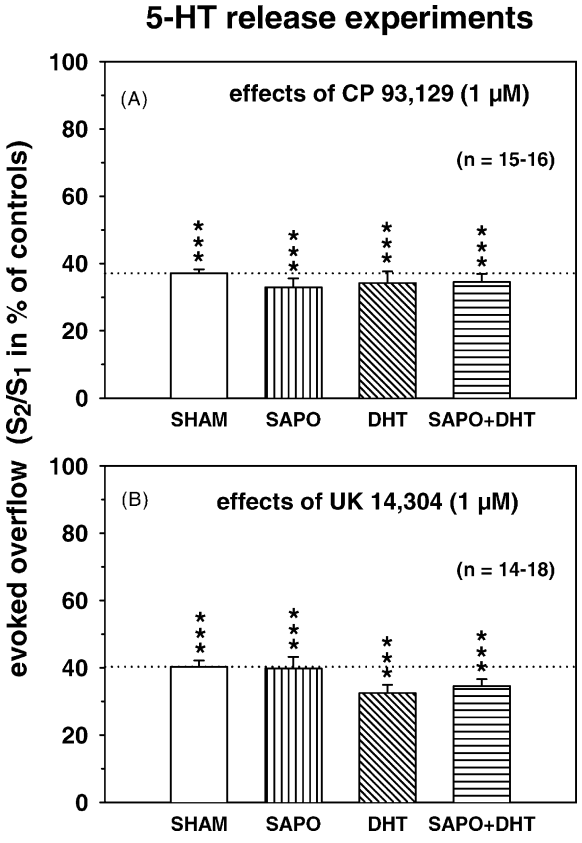

FIG. 5. Effect of the autoreceptor agonist CP 93,129 (A) and the α_2 receptor agonist UK 14,304 (B) on the electrically evoked overflow of [^{3}H]5-HT. Each drug was applied to the superfusate 12 min before S_2 at a concentration of 1 μM. Data are the mean ± SEM evoked overflow expressed as a ratio (S_2/S_1) and subsequently normalised by computing the percent of control values (no drug added to the superfusate). Data were from Sham-operated rats (SHAM), rats subjected to 192 IgG-saporin lesions (SAPO), to 5,7-DHT lesions (DHT) or to a combination of both lesions (SAPO + DHT). Number of rats per group: five to six; the minimal and maximal numbers of slices per column are indicated in the parentheses. *** Significantly different ($p < 0.001$) from the corresponding controls without exogenous agonists (see Section "RESULTS" for further differences).

Effects on [^{3}H]NA Release Induced by nAChR Stimulation

In separate experiments on untreated rats, the general properties of the release of [^{3}H]NA evoked by superfusion for 2 min with either 100 μM nicotine or 100 μM ACh (in presence of 1 μM physostigmine) were evaluated. Nicotine-evoked overflow of tritium was reduced to 12.9 ± 1.5% ($n = 8$; $p < 0.001$) of control values in the presence of 0.3 μM tetrodotoxin and to 32.7 ± 3.5% ($n = 8$; $p < 0.001$) of control values in the absence of extracellular calcium; moreover, it was diminished to 3.4 ± 0.8 ($n = 4$; $p < 0.05$) or to 4.5 ± 1.8% ($n = 4$; $p < 0.05$) of control values in the presence of 100 μM hexamethonium or 10 μM mecamylamine, respectively. The overflow of tritium evoked by 100 μM ACh

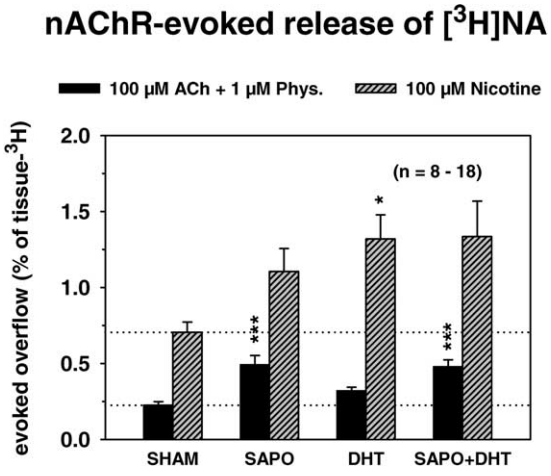

FIG. 6. ACh- (100 μM; solid bars) and nicotine-evoked (100 μM; hatched bars) release of [^{3}H]NA in slices from Sham-operated rats (SHAM), rats subjected to 192 IgG-saporin lesions (SAPO), to 5,7-DHT lesions (DHT) or to a combination of both lesions (SAPO + DHT). Number of rats per group: three to five; the minimal and maximal numbers of slices per column are indicated in parentheses. Data are the mean ± SEM evoked overflow expressed as a percent of tissue [^{3}H]. The ACh-evoked overflow was performed in the presence of 1 μM physostigmine. Significant differences from the corresponding values of SHAM rats: * $p < 0.05$; *** $p < 0.001$ (see Section "RESULTS" for further differences).

(in presence of 1 μM physostigmine) was reduced to 8.8 ± 1.1% ($n = 4$; $p < 0.01$) of control values in the presence of 0.3 μM tetrodotoxin and to 14.8 ± 2.2% ($n = 4$; $p < 0.01$) of control values in the absence of extracellular calcium; moreover, it was diminished to 6.5±0.9 ($n = 4$; $p < 0.01$) or to 6.6±1.7% ($n = 4$; $p < 0.001$) of control values in the presence of 100 μM hexamethonium or 10 μM mecamylamine, respectively. These observations suggest the involvement of nAChRs and of voltage-dependent sodium channels in this type of evoked overflow, as well as its (at least partial) dependence from the influx of calcium. Hence, this type of NA release may be characterised as evoked by stimulation of neuronal nicotine receptors, action potential-dependent and exocytotic.

Overall, the accumulation of [^{3}H]NA in the hippocampal slices of the various rat groups and the corresponding basal outflow of [^{3}H]NA was comparable to that found in the slices exposed to electrical field stimulation (see Figs. 1 and 3). Figure 6 shows the evoked relative overflow of NA in percent of accumulated [^{3}H] induced by either 100 μM ACh (in combination with 1 μM physostigmine; Fig. 6, solid bars), or by 100 μM nicotine (Fig. 6, hatched bars). The Kruskal–Wallis test showed that there was a significant difference among the four groups (KW = 19.44, $p < 0.001$) as concerns the release of NA induced by application of ACh in the presence of physostigmine. Dunn's test showed that rats from groups SAPO and SAPO + DHT exhibited a significantly higher release than rats from the group SHAM ($p < 0.001$ in both cases). Similar differences were found on absolute values (nCi; not illustrated). There was also a significant difference among the four groups on the relative release of NA induced by nicotine (KW = 7.68, $p = 0.05$), and this difference was due to values that were significantly higher in the group DHT as compared to the group SHAM ($p < 0.05$). Although the values in the groups with cholinergic lesions also exceeded those found in the SHAM rats, the differences were not significant. Analysis of absolute values (nCi) yielded similar differences (not shown). In all groups of rats, the ACh-evoked overflow of NA as well as that evoked by nicotine were reduced significantly in the presence of mecamylamine (10 μM; data not illustrated).

DISCUSSION

In the present study, intraseptal injections of 192 IgG-saporin were combined to lesions of serotonergic hippocampal afferents by injections of 5,7-DHT. 192 IgG-saporin lesions reduced the accumulation of [^{3}H]choline (−25%), the release of [^{3}H]ACh (−93%) and the activity of ChAT (−89%), increased the concentration of NA (+45%) and facilitated the ACh-evoked overflow of [^{3}H]NA in the hippocampus. 5,7-DHT lesions reduced the accumulation (−56%) and release (−13%) of [^{3}H]5-HT, the concentrations of 5-HT (−56%) and 5-HIAA (−56%), and facilitated the nicotine-evoked overflow of [^{3}H]NA. The accumulation and release of [^{3}H]NA were not altered by either lesion. The combination of both toxins resulted in a combination of their particular effects. CP 93,129 and oxotremorine reduced the release of [^{3}H]ACh in control and 5,7-DHT-lesioned rats (by about 30 and 70%, respectively). Because of a too weak release of [^{3}H]ACh, effects of these drugs could not be measured reliably in rats injected with saporin. CP 93,129 and UK 14,304 reduced the release of [^{3}H]5-HT in all groups (by about 65%).

Neurochemical Effects of the Lesions

The concentration of monoamines and the ChAT activity were measured in the ventral portion of the hippocampus, a portion not used for the release experiments.

Serotonergic lesions. In 5,7-DHT-lesioned rats, the concentrations of 5-HT and 5-HIAA were reduced by about 56%. These reductions were slightly smaller than described in previous reports ([1]: −75%; [36]: −70%), but appeared comparable to those found in a recent experiment [29]. The intraparenchymal injections of 5,7-DHT did not significantly alter the ChAT activity and had no effect on the concentration of NA, suggesting that the lesions showed some selectivity.

Cholinergic lesions. Consistent with previous reports, intraseptal injections of 192 IgG-saporin induced a dramatic depletion of ChAT activity (e.g. [2,37,47]). While these injections had no significant effect on the concentration of [5-HT] and [5-HIAA], they resulted in a 45% increase of the NA concentration. At least part of this increase probably reflected sprouting of sympathetic fibres, although one cannot exclude participation of central noradrenergic fibres, which also have a sprouting potential (e.g. [33]). Sympathetic sprouting is elicited by a cholinergic denervation of the hippocampus and accounts for a protracted increase of noradrenergic markers [15,27,33]. Harrell et al. [24] recently showed that such sprouting was also detected in the hippocampus of rats subjected to 192 IgG-saporin lesions.

Combined lesions. When 192 IgG-saporin and 5,7-DHT were administered in combination, the particular cholinergic and serotonergic effects were combined. These observations are in line with experiments in which 5,7-DHT lesions were combined to lesions damaging cholinergic nuclei without selectivity [36,38,41,42]. They also extend our previous studies in which the toxins were infused in the lateral ventricles [30] or the septal region [29]. Thus, these data provide further experimental arguments for using combinations of selective neurotoxins to study the functional correlates of interactions between various systems of neurotransmission.

Neuropharmacological Effects of the Lesions

Under the electrical stimulation conditions used herein, the electrically evoked overflow of tritium by slices preloaded with [^{3}H]choline, [^{3}H]5-HT or [^{3}H]NA was repeatedly shown to be Ca^{2+}-dependent and sensitive to tetrodotoxin. Therefore, it can

be considered as representing action potential-induced exocytotic neurotransmitter release (e.g. [22,23]). Our neuropharmacological data will be discussed in relation with the release of each of these transmitters, starting with 5-HT.

Uptake and release of [^{3}H]5-HT in hippocampal slices. In line with HPLC data, evidence for 5,7-DHT-induced serotonergic denervation was also reflected in hippocampal slices, where the accumulation and both basal and electrically evoked overflow of [^{3}H]5-HT were decreased. Although the accumulation of [^{3}H]5-HT certainly resulted from specific uptake (by axon terminals), as well as from nonspecific binding (e.g., to cell membranes), one may accept that, in a way or another, part of it was linked to the density of the serotonergic terminals in the hippocampus, the functional status of their uptake sites and/or their storage capacity. These remarks also apply to the other two transmitters investigated (ACh, NA). As already obvious from the concentrations of [5-HT] and [5-HIAA], the lesions were not complete and may thus have been followed by some sprouting of spared fibres [18,51]. If so, spared and sprouted fibres may both have contributed to a significant release of [^{3}H]5-HT in response to electrical field stimulation. This release was nevertheless weaker than in the Sham-operated rats, suggesting that the lesion-induced deficit was not compensated for when the experiment was run. When one compares this reduction (absolute: −57%; relative: −13%) to that found in previous studies (absolute: −62%; relative: +32%; [43]) after massive hippocampal denervation (e.g., by aspiration of the fimbria-fornix pathways), the effects of selective lesions seemed somewhat different from those obtained with extensive and unspecific lesion methods.

The release of [^{3}H]5-HT was almost not altered by the nearly complete damage of the cholinergic fibres innervating the hippocampus. While this observation is in line with the fact that there is no evidence for a role of nicotinic receptors in the release of this neurotransmitter (e.g. [45]), it is a bit surprising in the light of data showing that the release of 5-HT may be under the inhibitory influence of presynaptic M_1 receptors [34]. The latter conclusion was drawn from results obtained on synaptosomes using K^+ stimulations, and thus, under experimental conditions which cannot be compared to our present ones. Furthermore, as underlined by Vizi and Kiss [45], the fact that activation of a receptor that is usually excitatory inhibited the release of 5-HT may seem astonishing. Interestingly, in SAPO + DHT rats, the decrease in the accumulation and the release of [^{3}H]5-HT was not as pronounced as in DHT rats, and, although weak, this difference was significant for both variables. As the accumulation and the release of [^{3}H]5-HT were not facilitated in SAPO rats, the interpretation of this difference cannot be reduced to a simple effect of the cholinergic denervation alone. Interactions between cholinergic and serotonergic lesions, perhaps *via* functional alterations of terminals belonging to other transmitter systems, might have taken part in this phenomenon, but such an issue requires further experimental support.

Uptake of [^{3}H]choline and release of [^{3}H]ACh in hippocampal slices. A cholinergic denervation was also found in the slices from SAPO and SAPO + DHT rats, where accumulation of [^{3}H]choline and release of [^{3}H]ACh were reduced by more than 90%. This dramatic decrease, which prevented a reliable investigation of presynaptic modulatory mechanisms, was very comparable to that found in a recent experiment investigating the effects of intrahippocampal grafts rich in cholinergic neurons after intraseptal injections of 192 IgG-saporin [6]. Under normal conditions, the modulation of the release of ACh by cholinergic terminals in the hippocampus involves, among others, muscarinic, presumably M_2 autoreceptors [50], and 5-HT_{1B} heteroreceptors [45]. Previous experiments had shown that following lesions to the cholinergic innervation of the hippocampus, for instance by transsection of the fimbria-fornix, the amount of M_2 immunoreactivity in the hippocampus was reduced [31], an observation suggesting an alteration of possible substrates of presynaptic inhibition. It is, however, noteworthy that these findings were not confirmed after 192 IgG-saporin lesions [31]. Already in our recent experiment [6], the release of [^{3}H]ACh could not be measured reliably in the presence of oxotremorine or CP 93,129. However, in the few slices exhibiting some signs of release triggered by an electrical field stimulation, we found that the muscarinic and the 5-HT_{1B} receptors responded "normally" to their respective agonists [6].

Another point deserving discussion concerns the evoked overflow of [^{3}H]ACh in rats given 5,7-DHT lesions. As stated above, 5-HT may reduce the release of ACh by activating 5-HT_{1B} heteroreceptors located on cholinergic terminals [45]. In the rats given 5,7-DHT lesions, this reduction should be weaker and one might therefore expect some facilitation of the electrically evoked [^{3}H]ACh release. This was found in a recent experiment [7] in which 5,7-DHT was injected into the lateral ventricles, inducing an extensive serotonergic denervation of the hippocampus; the concentrations of 5-HT and 5-HIAA were reduced by more than 90% in the ventral hippocampus. Interestingly, this lesion-induced facilitation was counterbalanced by intrahippocampal grafts rich in serotonergic neurons. Why was a similar facilitation of evoked ACh release not found in the present experiment? We think that the reason is related to the extent of the serotonergic denervation: while it was massive in the study by Birthelmer et al. [7], it was only partial in the present one, thereby enabling the release of enough 5-HT to exert a sufficient activation of the 5-HT_{1B} heteroreceptors. This interpretation finds some support in the fact that, using the same stimulation conditions, the 5,7-DHT lesions reduced the relative evoked overflow of [^{3}H]5-HT by 13% in the present study as compared to 47% in the study by Birthelmer et al. [7].

Uptake of [^{3}H]NA and release of [^{3}H]NA in hippocampal slices. The release of [^{3}H]NA was studied in response to electrical field stimulation or to application of ACh or nicotine acting on nAChRs. The ACh- or nicotine-evoked overflow was sensitive to nicotinic antagonists (hexamethonium and mecamylamine) and—as for electrically evoked overflow of [^{3}H]NA—Ca^{2+}-dependent and TTX sensitive, indicating that following stimulation of nAChRs action potentials are induced which lead to exocytotic release of the neurotransmitter. The nAChRs involved in this effect might belong mainly to the $\alpha 3\beta 4$ type (see [46]), but possible involvement of the $\alpha 7$ subtype has also been proposed (see below).

As regards the data from selectively lesioned rats, several issues require discussion. First, the accumulation, basal and electrically evoked overflow of [^{3}H]NA were not altered by either lesion. This accounts for the fact that our cholinergic and serotonergic lesion methods, whether used separately or in combination, did not alter the noradrenergic innervation of the hippocampus, or at least not irreversibly. Second, while the concentration of NA was increased by the cholinergic lesion, the accumulation of [^{3}H]NA was not affected. This is not necessarily a contradiction as we have shown [27] that an increased concentration of NA (as compared to normal values) due to sympathetic sprouting can be detected several months before an above normal increase of NA uptake (in this case by hippocampal synaptosomes) is measured. In addition, sympathetic sprouting may be more pronounced in the ventral than in the dorsal hippocampus [27]: while the NA concentration was measured in a ventral portion of the hippocampus, the accumulation of [^{3}H]NA was determined in slices prepared from the dorsal two thirds of this structure. A third point concerns the fact that electrically evoked overflow of [^{3}H]NA was not altered by the lesions, in contrast to ACh- or nicotine-evoked overflow of [^{3}H]NA. It is well known that the release of NA can be triggered by activation of presynaptic nAChRs [45,46]. So far, inhibition of NA release by activation of presynaptic muscarinic receptors is

not documented consistently and its modulation by serotonergic mechanisms requires further studies before clear statements can be made [45]. However, given the role of presynaptic nAChRs in NA release, it seemed reasonable to expect that the release of NA would be deprived of its nicotinic component following the almost complete cholinergic denervation of the hippocampus. This was not the case, and an explanation might be that the *electrical* stimulation conditions were not appropriate to detect lesion-induced alterations in the nicotinic regulation of NA release.

Fortunately, however, lesion-induced changes in this regulation could be observed in the experiments using *chemical* stimulation of the [^{3}H]NA release (Fig. 6). Here, we found that the cholinergic lesion facilitated the release of [^{3}H]NA triggered by application of ACh, and that the serotonergic lesion (not the cholinergic one) surprisingly facilitated the release triggered by application of nicotine. While the effects of ACh might be explained by a lesion-induced upregulation of the nicotinic receptors, it is not clear why this facilitation did not reach significance in the presence of nicotine. However, as the release also tended to be increased in SAPO and SAPO + DHT rats, it seems reasonable to attribute this failure to too small sample sizes and to replicate this experiment on a larger number of rats before furthering the discussion on this point.

Even more enigmatic—at first view—is the significant facilitation of [^{3}H]NA release evoked by application of nicotine in the 5,7-DHT-lesioned rats, a result suggesting that some mechanisms related to this nicotine-evoked overflow of [^{3}H]NA involve a serotonergic inhibitory control. Such an inhibitory mechanism seems, however, to exist. For instance, Hennings et al. [25] have reported that monoamine uptake blockers inhibited the nicotine-evoked overflow of [^{3}H]NA, and that the underlying mechanism did not involve the neuronal NA transporter. Hennings et al. [25] also reported that fluoxetine, a specific 5-HT reuptake inhibitor, produced similar effects, but these were attributed to nicotinic antagonist properties of the drug. Although the question of the subtype of the nAChR involved in the nicotine-evoked overflow of [^{3}H]NA is still subject to debate [45,46], it is noteworthy that alpha-bungarotoxin, an α7-specific nAChR antagonist, attenuates the nicotine-evoked overflow of [^{3}H]NA in hippocampal slices [20]. Moreover, recent results obtained with human neuronal α7 nAChRs suggest that 5-HT is able to act on α7 receptors as an antagonist [21]. If a similar mechanism would work in rats, one might propose that the facilitated nicotine-evoked overflow of [^{3}H]NA in the rats with 5,7-DHT lesions is due to a reduced inhibitory influence of 5-HT on nAChRs. If so, the tendencies towards a facilitated release found in SAPO and that found in DHT rats might involve different mechanisms, one being based on a cholinergic lesion-induced upregulation of nicotinic receptors, the other one being based on a serotonergic lesion-induced disinhibition of the nAChR. These speculations invite to further exploration of the issue.

In conclusion, our results confirm that selective neurotoxins can be combined to enable controlled and selective damage to a multitude of transmitter systems. Compared with the literature and previous studies, they also suggest that, under the experimental conditions used in this study, (i) partial serotonergic lesions do not influence the release of ACh at the presynaptic level by a reduced serotonergic activation of the inhibitory 5-HT$_{1B}$ heteroreceptors, (ii) in the presence of an intact noradrenergic innervation, spared serotonergic terminals do not upregulate their release capabilities, probably because this upregulation only appears when the noradrenergic fibres are also damaged and do not longer inhibit the serotonergic terminals and (iii) 5-HT might exert an inhibitory influence on the nAChR-evoked overflow of NA. Finally, our results show that presynaptic modulatory mechanisms involving auto- and heteroreceptors, when measurable (in case of massive denervation their determination is not longer reliable), may be conserved on fibres spared by the lesions.

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