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Reduction in Acetylcholine Release in the Hippocampus of Dopamine D5 Receptor-Deficient Mice

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Activation of the dopamine D_1-like receptor stimulates acetylcholine (ACh) release in the hippocampus, apparently through the molecularly defined d5 receptor. In the present study, we used a transgenic mouse completely deprived of functional d5 receptor (d5−/−) to confirm the role and elucidate the possible function of the d5 receptor subtype on hippocampal cholinergic neurotransmission. ACh release was measured using *in vivo* microdialysis in the mouse dorsal hippocampus of 4 months old homozygous (d5−/−), heterozygous (d5+/−), and the wild-type (d5+/+) littermates. Using the no net flux technique, a significant reduction in basal hippocampal ACh level was found in the d5−/− compared to d5+/− and d5+/+ mice. Moreover, the administration of SKF 38393, a D_1-like receptor agonist, systemically (2.0 and 10.0 mg/kg ip), or locally through the dialysis probe (10 and 50 μM), produced a dose-dependent enhancement of ACh release in the d5+/+, a moderate stimulation in the d5+/− but had no effect in the d5−/− mice. Quantitative receptor autoradiography revealed significant increases in M_1-like but not in M_2-like muscarinic receptor binding sites in the hippocampal formation. These results confirm and extend the role of the d5 receptor in the modulation of hippocampal ACh release and provide evidence for long-term alteration of hippocampal cholinergic neurotransmission resulting from the absence of the d5 receptors including chronically reduced ACh release and change in M_1-like receptor levels.
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INTRODUCTION

Dopamine (DA) exerts a modulatory role on acetylcholine (ACh) release in the hippocampus (Day *et al*, 2001; Hersi *et al*, 1995a, b). Interactions between these two neurotransmitter systems play key roles in learning and memory (Levin *et al*, 1990) and these systems, particularly the cholinergic innervation, are severely affected in Alzheimer's Disease (Araujo *et al*, 1988; Auld *et al*, 2002; Davies and Maloney, 1976; Perry *et al*, 1992). Hippocampal cholinergic nerve terminals originate from the medial septum and the vertical limb of the diagonal band of Broca and project to the hippocampal formation via the fimbria fornix (Mesulam *et al*, 1983; Woolf, 1991). The hippocampus also receives dopaminergic projections arising mainly from the ventral tegmental area with little contribution of the substantia nigra, pars compacta (Gasbarri *et al*, 1994; Verney *et al*, 1985).

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Numerous studies demonstrated that DA stimulates hippocampal ACh release through the activation of D_1-like receptors(Day and Fibiger, 1994; Imperato *et al*, 1993), most probably through receptors located on hippocampal cholinergic nerve terminals (Hersi *et al*, 1995a). Moreover, behavioral studies revealed that peripheral administration of a D_1-like agonist improved cognitive performance in rats (Steele *et al*, 1996, 1997), reduced age-related deficits in spatial memory in mice (Bach *et al*, 1999), and attenuated learning deficits in aged memory impaired rats possibly through the stimulation of hippocampal ACh release (Hersi *et al*, 1995b).

The D_1-like receptor subfamily includes the molecularly defined d1 and d5 subtypes (Dearry *et al*, 1990; Sunahara *et al*, 1990, 1991; Zhou *et al*, 1990), which are both expressed in the hippocampus (Bergson *et al*, 1995; Tiberi *et al*, 1991). These two subtypes share approximately 50% sequence homology (80% in the transmembrane domains), which translates into a highly similar pharmacological profile (Seeman and Van Tol, 1993; Sunahara *et al*, 1991; Tiberi *et al*, 1991). No drugs are currently able to discriminate between d1 and d5 receptors. Recently, an antisense oligonucluotide approach revealed that the d5 (but not d1) receptor was the subtype likely involved in the modulation of hippocampal ACh release (Hersi *et al*, 2000).

In the present study, we used an *in vivo* microdialysis technique in mice lacking functional d5 receptors (d5−/−) to elucidate further the role of the receptor subtype in hippocampal ACh release. The results reported here confirm the tonic role of the d5 receptor in the dopaminergic modulation of hippocampal ACh release and demonstrated long-term alterations in M_1-like muscarinic receptors in the knockout animal model.

MATERIALS AND METHODS

Materials

The d5−/− mouse with a genetic background of 129/SvJ1 and C57BL/6J was generated as described earlier (Hollon *et al*, 2002) and provided by one of the authors (D Sibley). Immunohistochemical detection of d5 receptors revealed that the frontal cortex in the d5−/− mouse was, as expected, completely devoid of this receptor (Hollon *et al*, 2002). Animals were reproduced in our animal facilities by standard inbreeding. Females were isolated to give birth and pups were weaned at postnatal day 21. The genotype of each mouse was determined using a PCR technique as previously described (Hollon *et al*, 2002). Experiments were performed in homozygous d5−/−, heterozygous (d5+/−), and wild-type (d5+/+) littermates. Homozygous d5−/− mice are viable, develop normally and are fertile and capable of reproduction (Hollon *et al*, 2002). These animals were housed two to six per cage at room temperature and on a 12-h light/dark schedule. The animals were fed *ad libidum* with standard lab chow and tap water up to the time of the experiments, according to protocols and guidelines approved by the McGill University Animal Care Committee and the Canadian Council for Animal Care (CCAC).

Ketamine, xylazine, and acepromazine were obtained from Vetrepharm (Belleville, ON), Novopharm (Toronto, ON), and Wyert-Ayerst (Montréal, PQ), respectively. The dialysis membrane used was made from AN69 Hospal Industry (molecular weight cut off 60 000 Da, i.d. = 0.22 mm, o.d. = 0.31 mm; Hospal, Lyon, France). The membrane was covered with epoxy glue along its whole length except for 4 mm corresponding to the area of dialysis. 1-Phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol (SKF 38393) and neostigmine bromide were obtained from Sigma RBI (Oakville, ON).

The microliter syringe pump was from Harvard Apparatus Inc. (South Natick, MA), ESA 580 HPLC dual piston pump from ESA (Chelmford, MA), CMA 260 degasser from Cargenie Medicin (Stockholm, Sweden), Lichrosorb NH_2 (10 μm) was from Merck (Darmstadt, Germany), platinum and Ag/AgCl reference electrodes (Antec VT-03/Decade) from Decade (Leiden, the Netherlands), and column hardware and packing materials were from Chrompack (Raritan, MA). [^{3}H]SCH 23390 (73.0 Ci/mmol), [^{3}H]pirenzepine (79.3 Ci/mmol), and [^{3}H]AFDX-384 (137.0 Ci/mmol) were from New England Nuclear (Boston MA) and tritium-sensitive films from Amersham (Oakville, ON).

All other reagents and chemical for the *in vivo* microdialysis, HPLC, and receptor autoradiography were obtained from Sigma Chemical Co (St Louis, MO).

Probe Implantation

The microdialysis probe was implanted in the dorsal hippocampus according to procedures described by our group for rats with minor modifications (Day *et al*, 2001; Hersi *et al*, 1995a; Kitaichi *et al*, 1999). Male mice, 4-month old, were anesthetized using a mix of ketamine (100 mg/kg), zylazine (20 mg/kg), and acepromazine (10 mg/kg) administered intramuscularly. Animals were placed in a stereotaxic apparatus. The skull was uncovered and lateral muscle displaced to expose parietal bone. One hole (about 2 mm diameter) was drilled on each side. The dialysis transverse probes were inserted through the holes at the level of the dorsal hippocampus, 2.2 mm posterior to bregma and 2.0 mm below the surface of the skull (Franklin and Paxinos, 1997). After insertion, the ends of the dialysis tube were connected to a stainless steal cannulae using epoxy glue. Steel cannulae were secured on the top of the skull using dental cement and fixed on the skull with a screw. *In vivo* microdialysis were performed 48 h after surgery.

In Vivo Microdialysis

At the beginning of each dialysis experiment, animals were placed in a lidless cage and the probes connected to a microliter syringe pump in a manner as to allow mice to move freely in the cage. The dialysis probe was perfused at a constant rate of 2.5 μl/min with a cerebrospinal fluid-like solution containing (in mM) NaCl 123, KCl 3, $CaCl_2$ 1.3, $MgCl_2$ 1 $NaHCO_3$ 23, and sodium phosphate buffer 1 pH 7.4. Neostigmine bromine (10 nM), an acetylcholinesterase inhibitor, was added to the solution. After a 1 h washout period, 20 min dialysate fractions were collected. SKF 38393 (2.0 and 10.0 mg/kg in sodium chloride 0.9% solution) was administered by intraperitoneal (i.p.) injection or locally (10 or 50 μM) through the dialysis probe. Each animal was dialysed once and tested for only one pharmacological treatment.

No Net Flux Method

The no net flux microdialysis technique is used to determine the *in vivo* extracellular concentration of a neurotransmitter (Day *et al*, 2001; Justice, 1993). Another group of animals was perfused with the same cerebrospinal fluid-like solution used in the former experiment without neostigmine bromine but containing a known concentration of ACh (2.5, 5.0, 7.5, or 10.0 nM) [ACh_{in}] perfused through the dialysis tube at a constant rate of 5 μl/min. When [ACh_{in}] is lower than that surrounding the probe, ACh will flow down its concentration gradient into the probe, increasing the resultant ACh concentration measured in the dialysate [ACh_{out}]. When [ACh_{in}] is higher than that surrounding the probe, ACh will flow out of the probe and [ACh_{out}] will be smaller than [ACh_{in}]. By plotting [ACh_{in-out}] as a function of [ACh_{in}], the point where the line cross the *x*-axis can be extrapolated (ie where [ACh_{in-out}] = 0, where there is no net flux) to give a measure of [ACh] surrounding the probe. For this experiment, dialysate samples were collected every 20 min and assayed for ACh content.

ACh Measurement

ACh content in dialysate fractions was determined using HPLC separation, postcolumn enzymatic reaction, and electrochemical detection (Day *et al*, 2001). ACh was separated from other molecules in the dialysate on a reverse phase column (75 × 2.1 mm) pretreated with sodium lauryl sulfate. From this column, the eluate then passes through an enzyme reactor (10 × 2.1 mm) containing acetylcholinesterase (AChE; EC 3.1.1.7; type VI-S) and choline oxidase (EC 1.1.3.17) covalently bound to gluteraldehyde-activated Lichrosorb NH_2. The separated ACh reacts with the enzymes to give stoichiometric yield of hydrogen peroxide, which is electrochemically detected with a platinum electrode at a potential of +500 mV *vs* an Ag/AgCl reference electrode. The mobile phase consist of a 0.2 M aqueous potassium phosphate buffer, pH 8.0, containing 1 mM tetramethylammonium hydroxide and is delivered at 0.35–0.45 ml/min and is degassed online. ACh elutes at ~4 min, and the best detection limit of the assay is ~10 fmol/injection. Sample concentrations were calculated by comparison with known standards.

Receptor Autoradiography

Animals were killed by decapitation. Brains were removed and frozen in 2-methylbutane at −40°C and stored at −80°C. Coronal brain sections (20 μm) were cut at −18°C on a cryostat, thaw-mounted on gelatin-coated slides and stored at −80°C until use.

D1-like DA receptors. D1-like receptor binding sites were visualized using [^{3}H]SCH 23390 as described before (Flores *et al*, 1996). Brain sections were preincubated for 10 min at room temperature in buffer containing 50 mM Tris-HCl, pH 7.4, 154 mM NaCl, 1 mM EDTA, and 0.1% of bovine serum albumin. Sections were incubated at room temperature in the same buffer containing 2 nM [^{3}H]SCH 23390 and 30 nM ketanserin to mask possible binding of the ligand to serotonergic 5-HT2 sites. Consecutive sections were also incubated in presence of 1 μM (+)-butaclamol to ascertain the specificity of the binding. Incubations were terminated by dipping slides into ice-cold buffer followed by two consecutive 10 min washes in buffer and a rapid dip into ice-cold distillated water. Slices were air-dried and autoradiograms were generated by apposing sections alongside tritium standards to tritium-sensitive films for 15 days.

Muscarinic receptors. Muscarinic M_1-like and M_2-like receptor binding sites were visualized using [^{3}H]pirenzepine and [^{3}H]AFDX-384, respectively, as described elsewhere (Vaucher *et al*, 2002). Brain sections were preincubated for 10 min in Krebs buffer (NaCl 120 mM; KCl 4.7 mM; $CaCl_2$ 2.5 mM; KH_2PO_4 1.2 mM; $MgSO_4$ 1.2 mM; glucose 5.6 mM; $NaHCO_3$ 25 mM, pH 7.4) at room temperature before a 60-min incubation into the same buffer containing either 10 nM [^{3}H]pirenzepine or 2 nM [^{3}H]AFDX-384. Consecutive sections were incubated in the presence of atropine (1 μM) to ascertain the specificity of the labelling. Sections were rinsed three times (4 min each) in ice-cold Tris-HCl buffer (50 mM, pH 7.4) followed by a rapid dip in ice-cold distilled water to removed buffer salts and sections were air-dried. Autoradiograms were generated by apposing sections alongside tritium standards to tritium-sensitive films for 15 days for [^{3}H]pirenzepine binding and 3 weeks for [^{3}H]AFDX-384 binding.

Films were developed as described before (Quirion *et al*, 1981) and the specific labelling was quantified (fmol/mg tissues wet weight) using a computer-assisted microdensitometric image analysing system (MCID System, Imaging Research Inc., St Catherines, Ontario, Canada).

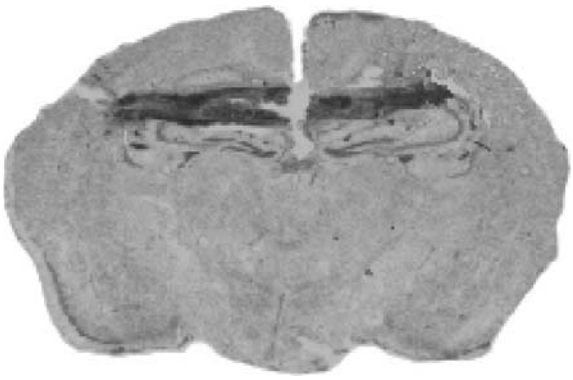

Figure 1 Histological representation of a coronal section from a 4-month old mouse brain stained with cresyl violet demonstrating the location of the microdialysis probe into the dorsal hippocampus.

Histology

After each *in vivo* microdialysis experiment, animals were killed by cervical dislocation and brains were removed and frozen in 2-methylbutane at −40°C and stored at −80°C. Then, 20 μm coronal sections were mounted onto gelatin-coated slides and stained with cresyl violet to confirm probe location into both dorsal hippocampi (Figure 1). Animals whose probe was not implanted in the appropriate area were excluded from the study.

Statistical Analysis

For *in vivo* microdialysis experiments, data were analyzed by a repeated measure three-way analysis of variance (ANOVA), for the main effect of genotype, main effect of pharmacological treatments, main effect of time as repeated measures, and interaction between these three factors. *Post hoc* Bonferonni analyses were conducted when appropriate. For the no net flux experiments, basal ACh release and for receptor autoradiography, significant differences between experimental groups were determined using one-way ANOVA with *post hoc* Dunnett's multiple comparisons with the d5+/+ genotype, $p<0.05$ being considered significant.

RESULTS

Determination of Basal Hippocampal ACh Level

Using the no net flux technique, we established the extracellular concentration of ACh into the dorsal hippocampus to 4.4±0.2 nM for the d5+/+ ($n=11$), 4.0±0.2 nM for d5+/− ($n=7$), and 2.8±0.1 nM for the d5−/− ($n=11$) mice (Figure 2). A significant reduction of

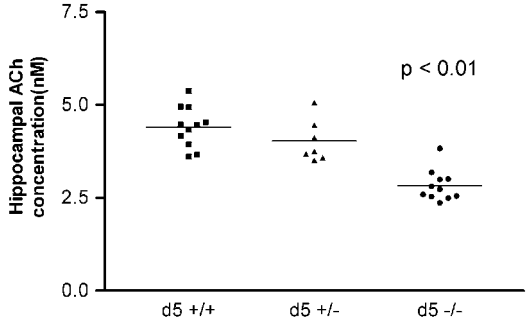

Figure 2 Extracellular levels of ACh as determined using the no net flux method in dopamine d5 knockout (d5−/−) heterozygous (d5 + /−) and wild-type (d5 + / +) mice. Each point represents individual animals. *Post hoc* analysis revealed significant differences between d5 + / + and d5−/− ($p < 0.01$).

basal hippocampal ACh level was hence observed in d5−/− mice compared to d5 + / + controls ($F_{(2,26)} = 28.43$, $p < 0.01$), whereas d5 + /− had no significant reduction compared to the wild-type mice.

Before pharmacological treatments, the average basal efflux of ACh was 274 ± 28 fmol/50 µl dialysate in d5 + / + ($n = 31$), 239 ± 19 fmol/50 µl dialysate in d5 + /− ($n = 29$), and 180 ± 18 fmol/50 µl dialysate in d5−/− ($n = 30$). In accordance with data obtained using the no net flux method, a significant reduction of basal hippocampal ACh release was observed in d5−/− mice compared to d5 + / + controls ($F_{(2,89)} = 4.44$, $p = 0.015$).

Effect of SKF 38393, a D_1-like Agonist

Three-way ANOVA revealed significant interaction between the main effects of genotype, treatment, and time ($F_{(44,539)} = 2.70$, $p < 0.0001$). Saline administration slightly increased ACh release likely as a consequence of the animal handling. However, no significant differences in saline effects were observed between the three animal genotypes (Figure 3a). The systemic administration of SKF 38393 (2.0 and 10.0 mg/kg ip) differentially affected ACh release in the hippocampus of the animal subgroups studied here. The D_1-like agonist increased hippocampal ACh release to a greater extent at 20, 40, and 60 min, postinjection in d5 + / + compared to d5 + /− and d5−/− mice at both 2.0 mg/kg (Figure 3b) ($p < 0.0001$) and 10.0 mg/kg (Figure 3c) ($p < 0.0001$).

Moreover, systemic administration of SKF 38393 at both doses significantly increased hippocampal ACh release in d5 + / + mice compared to saline injection ($p = 0.0005$, 2.0 mg/kg; $p < 0.0001$, 10.0 mg/kg). In d5−/−, the administration of SKF 38393 did not increase hippocampal ACh release compared to saline. In d5 + /−, a significant increase in ACh release was observed only following the administration of SKF 38393 at the higher dose used here ($p < 0.0001$) (Figure 3c).

Local perfusion of SKF 38393 (10 and 50 µM) directly through the dialysis probe significantly increased ACh release to 200 and 290% over basal values in the wild-type mice (Figure 4a, b), but only over 140 and 150% in d5 + /− mice (Figure 4a, b) while having no effect d5−/− mice (Figure 4a, b). Three-way ANOVA revealed a significant

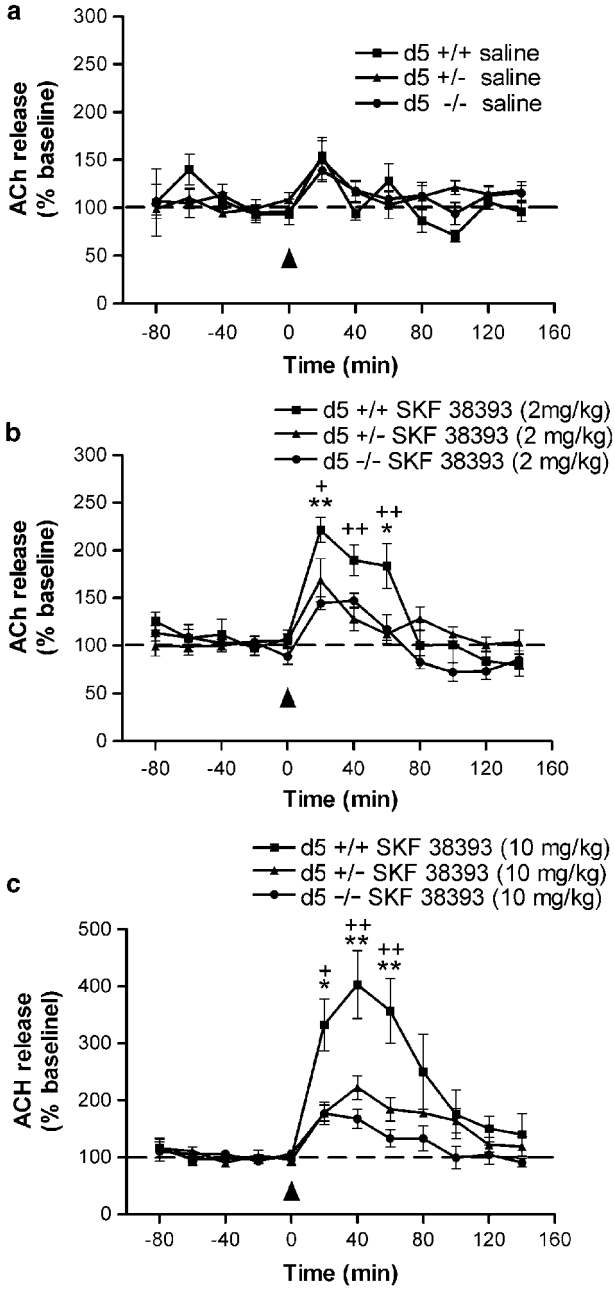

Figure 3 ACh release in the dorsal hippocampus in mice (d5−/−, d5 + /− and d5 + / +) following intraperitoneal administration of (a) saline solution, (b) SKF 38393 at 2.0 mg/kg, and (c) SKF 38393 at 10.0 mg/kg. Data represent mean ± SEM ($n = 6$–7 in each group). Dialysate ACh content is expressed as percent of baseline. Baseline was calculated from the average of three samples preceding drug injection (arrow). For both doses, *post hoc* analysis revealed significant differences of drug effect between d5 + / + and d5−/−: **$p < 0.001$; *$p < 0.01$ and between d5 + / + and d5 + /−: + $p < 0.05$; ++ $p < 0.01$.

interaction between the main effects of genotype, treatment, and time ($F_{(22,264)} = 2.26$, $p = 0.0001$). A significant difference in hippocampal ACh release following stimulation with SKF 38393 is observed between d5 + / + and d5−/− at both concentrations tested in this study ($p < 0.0001$ for both concentrations) and a significant difference is observed between d5 + /− and d5−/− only at 50 µM ($p = 0.002$).

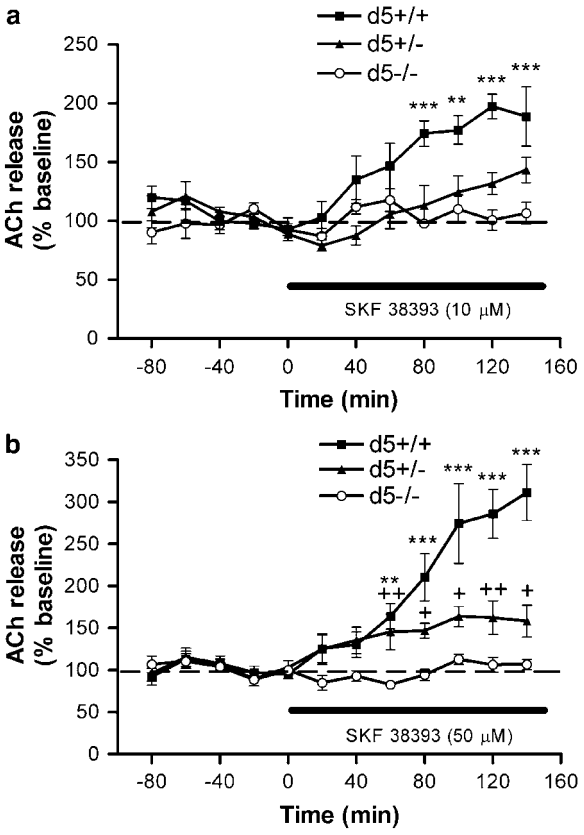

Figure 4 ACh release in the dorsal hippocampus in mice (d5−/−, d5 + /− and d5 + / +) following local perfusion of (a) 10 μM and (b) 50 μM SKF 38393. Data represent mean ± SEM ($n = 5$–6 in each group). Dialysate ACh content is expressed as percent of baseline. Baseline was calculated from the average of three samples preceding drug perfusion (line). *Post hoc* analysis revealed significant differences of the drug effect between d5 + / + and d5−/−: $^{**}p<0.01$; $^{***}p<0.001$ and between d5 + /− and d5−/−: $^{+}p<0.05$; $^{++}p<0.01$.

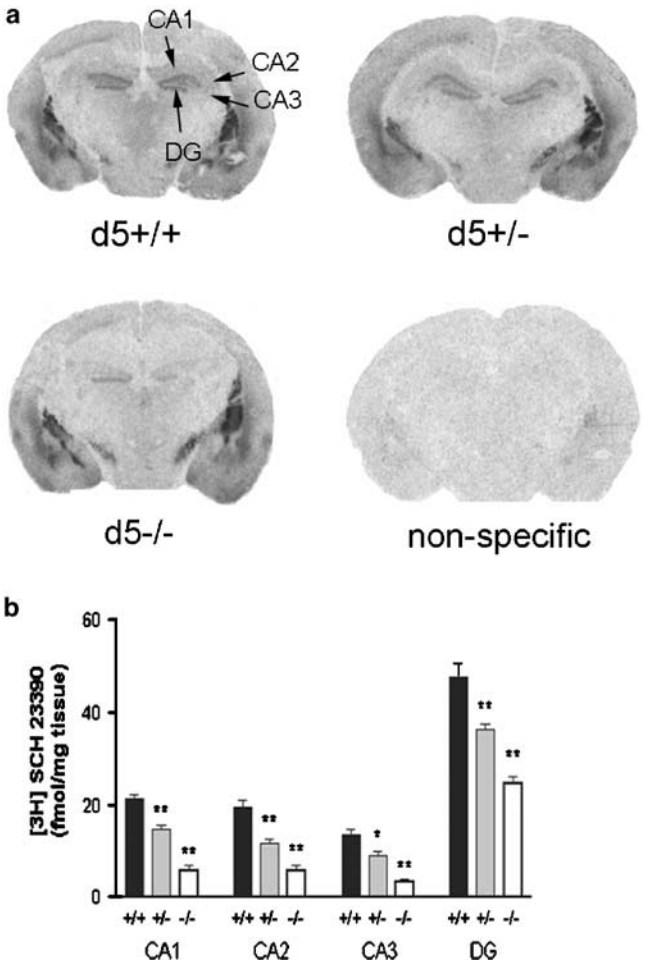

Figure 5 Quantitative autoradiography of D_1-like DA receptor binding sites at the level of the hippocampal formation. (a) D_1-like of dopaminergic receptors are known to be expressed in all subareas of the hippocampus. [^{3}H]SCH 23390 exhibited 80% of specific binding in the normal mice. (b) D_1-like receptors were quantified in all genotypes. Data represent mean ± SEM ($n = 6$–8 in each group). ANOVA with Dunnett's multiple comparisons revealed significant difference between d5 + / + and d5 + /− and between d5 + / + and d5−/−: $^{*}p<0.05$; $^{**}p<0.01$. Abbreviations: Ca1, Ca2, Ca3, subfields of the Ammon's horn of the hippocampus. DG, dentate gyrus.

Quantitative Receptor Autoradiography

Deletion of d5 receptors in d5−/− mice resulted in significant reductions in specific [^{3}H]SCH 23390 binding in all areas of the hippocampal formation (50% in the dentate gyrus and 73% in other regions) compared to wild-type animals (Figure 5a, b) ($p<0.01$). d5 + /− mice exhibited intermediate declines in specific [^{3}H]SCH 23390 binding (from 25 to 40% reduction; $p<0.05$ for CA3 subfield and $p<0.01$ for other regions) compared to wild-type animals.

Hippocampal [^{3}H]pirenzepine M_1-like and [^{3}H]AFDX-384 M_2-like muscarinic receptor binding sites are expressed in all subareas of the hippocampal formation (Figure 6a, c). Specific [^{3}H]pirenzepine binding is significantly increased in all subregions of the hippocampal formation in the d5 knockout mouse (Figure 6b). Heterozygote mice displayed moderate increases in specific [^{3}H]pirenzepine binding in the CA2 and CA3 subfields compared to d5 + / + (Figure 6b). Specific [^{3}H]AFDX-384 M_2-like binding sites was not significantly changed in the three groups studied here (Figure 6d).

DISCUSSION

The main findings of the present study are that extracellular levels of hippocampal ACh are chronically decreased, and that a DA D_1-like agonist failed to modulate this release in the hippocampus of d5 receptor knockout mice. A blunted, but still significant effect was observed in heterozygous mice (d5 + /−) while SKF 38393, the D_1-like agonist, potentially facilitated ACh release in the hippocampus of wild-type mice. These results confirm and extend previous data mostly obtained in the rat suggesting that the d5 receptor subtype was the D_1-like receptor involved in the regulation of hippocampal ACh release (Hersi *et al*, 1995a, 2000). The use of molecular approaches such as the one reported here based on knockout animals or oligonucleotide antisenses (Hersi *et al*, 2000) is required as currently available pharmacological tools cannot distinguish between d1 and d5 receptors, the two subtypes composing the D_1-like family of DA receptors (Seeman and Van Tol, 1993; Sunahara *et al*, 1991).

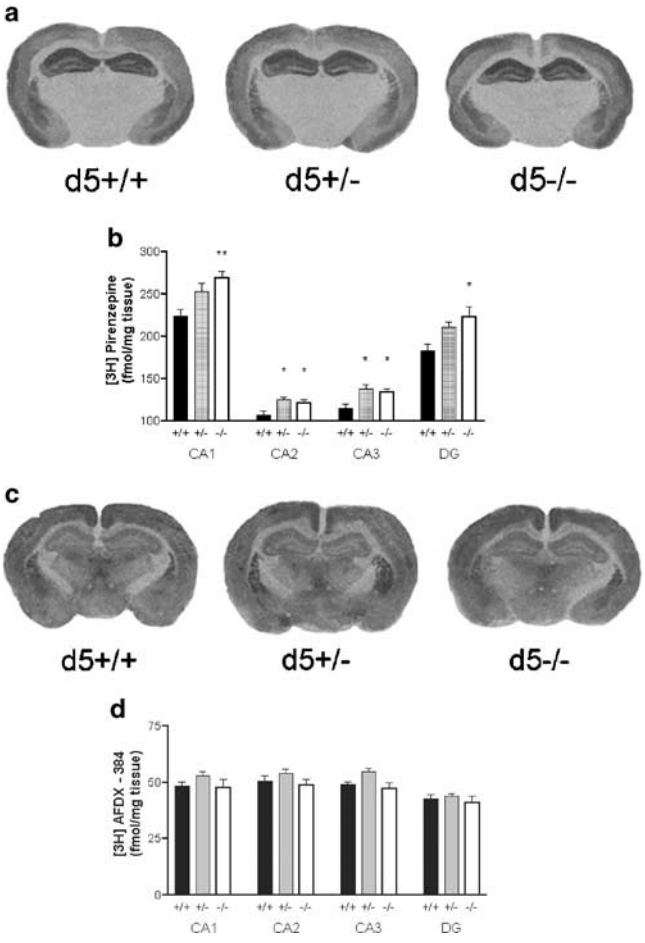

Figure 6 Quantitative autoradiography of muscarinic receptor binding sites in the hippocampal formation. Both M_1 and M_2-like muscarinic receptor classes are expressed in all subareas of the hippocampus. [^{3}H]pirenzepine and [^{3}H]AFDX 384 exhibited almost 100% of specific binding. (a, b) M_1-like and (c, d) M_2-like muscarinic receptor binding sites were quantified in all genotypes. Data represent mean ± SEM ($n = 7$ in each group). ANOVA with Dunnett's multiple comparisons revealed significant difference between d5+/+ and d5+/− and between d5+/+ and d5−/−: *$p < 0.05$; **$p < 0.01$. Abbreviations: Ca1, Ca2, Ca3, subfields of the Ammon's horn of the hippocampus. DG, dentate gyrus.

In the d5−/− mice, the lack of stimulatory effects of the D_1-like agonist SKF 38393 on hippocampal ACh release was observed following systemic injections or intraprobe infusions of the agonist. In the heterozygous mice, significant effects of SKF 38393 were seen only at the higher concentration tested in this study, while the D_1-like agonist potently facilitated *in vivo* hippocampal ACh release at both concentrations tested. These results are likely due to the loss of D_1-like receptors in the hippocampal formation of d5+/− and d5−/− mice. Indeed, we observed that specific [^{3}H]SCH 23390 D_1-like receptor binding levels were significantly decreased in d5 knockout and heterozygous mice. The residual labelling seen in these animals is likely reflecting the presence of low but significant levels of d1 receptors in the hippocampus (Bergson *et al*, 1995; Tiberi *et al*, 1991). However, this class of receptors does not appear to be involved in the direct regulation of hippocampal ACh release as we failed to observe any effects of SKF 38393 in d5−/− animals. A previous study reported no reduction in specific [^{3}H]SCH 23390 binding in the striatum; this is in accordance with the fact that this brain region is mostly enriched with d1 receptors (Hollon *et al*, 2002).

Anatomical evidence have shown that a subpopulation of hippocampal d5-like receptors are located on axons in the hippocampus (Bergson *et al*, 1995). Moreover, fimbriectomia resulted in significant losses in D_1-like receptors in the dentate gyrus of the hippocampus (Hersi *et al*, 1995a). These data may be taken as evidences that d5 receptors are present on septo-hippocampal cholinergic nerve terminals where they can directly modulate ACh release.

Using the technique of 'no net flux' *in vivo* microdialysis (Justice, 1993), the calculated level of extracellular ACh was in the range of 4.4 nM in the wild-type mouse dorsal hippocampus. This is comparable to the level reported in the rat dorsal hippocampus (Day *et al*, 2001). Interestingly, the basal level of ACh in the d5 knockout mouse (2.8 nM) was significantly lower than in heterozygous (4.0 nM) and wild-type (4.4 nM) mice. This result suggests that DA, acting though the d5 receptor, has a tonic role on the maintenance of normal ACh release in the hippocampus. Interestingly, the d5, but not d1, receptor subtype has been shown to be constitutively active even in the absence or in the presence of very low concentrations of DA (Tiberi and Caron, 1994; Demchyshyn *et al*, 2000). Such a constitutive activity could play a role in the maintenance of extracellular levels of ACh in the hippocampus.

What could be the functional relevance of hippocampal d5 receptors on the maintenance of normal release of ACh leading to enhanced extracellular levels? While it is well established that hippocampal cholinergic innervation plays a major role in attention and learning behaviors (Perry *et al*, 1999; Everitt and Robbins, 1997), the role of DA and dopaminergic receptors is not as clear, even if few studies have proposed that the D_1-like receptors can facilitate cognitive behaviors (Packard and White, 1991; Arnsten *et al*, 1994). In a recent study, Holmes *et al* (2001) reported normal spatial memory in d5−/− mice in the Morris water maze, except for a minor deficit in hidden platform acquisition. Similar small deficits were seen in fear conditioning (Holmes *et al*, 2001). However, studies using pharmacological approaches have reported that D_1-like agonists can facilitate the late phase of long-term potentiation (LTP) and restore memory impairments in aged mice (Bach *et al*, 1999), and aged memory-impaired rats (Hersi *et al*, 1995b). Behavioral tests performed in d5−/− mice involved learning to avoid aversive stimuli (Holmes *et al*, 2001). It would thus be appropriate to investigate further the abilities of d5−/− mice in attention and learning models that are more discriminating for various aspects of the cognitive process, such as reward-related memory mechanisms (Schultz *et al*, 1993). Also, a particular focus could be on attention as multiple studies have suggested a role for DA in attention behaviors over memory processes *per se* (Clark *et al*, 1987a, b; Nieoullon, 2002).

In addition to changes in extracellular ACh levels, increases in muscarinic M_1-like, but not M_2-like, receptors were found in the hippocampus of d5−/− mice. M_1-like receptors are mostly postsynaptically located in the hippocampus and are believed to be involved in LTP and in the maintenance of normal cognitive functions

(Anagnostaras *et al*, 2003; Quirion *et al*, 1989). The increase in M_1-like receptors may be a compensatory mechanism associated to the decrease in extracellular ACh levels found in this brain region in d5 knockout mice. This compensatory mechanism could also possibly explain the apparent lack of learning deficits of d5−/− mice in the Morris water maze task (Holmes *et al*, 2001). On other hand, M_2-like receptors are mostly presynaptic auto receptors exerting inhibitory effect on ACh release (Lapchak *et al*, 1989; Quirion *et al*, 1989).

In summary, deletion of the d5 receptor in a knockout mouse model results in a significant reduction in extracellular ACh levels in the hippocampal formation, suggesting a tonic role for this receptor in ACh release. Moreover, a D_1-like agonist fails to stimulate *in vivo* ACh release in this model demonstrating further the unique role of the d5 receptor in the regulation of hippocampal ACh release. Further studies are now in progress to establish more precisely the behavioral relevance of these findings.

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REFERENCES

Anagnostaras SG, Murphy GG, Hamilton SE, Mitchell SL, Rahnama NP, Nathanson NM *et al* (2003). Selective cognitive dysfunction in acetylcholine M1 muscarinic receptor mutant mice. *Nat Neurosci* **6**: 51–58.

Araujo DM, Lapchak PA, Robitaille Y, Gauthier S, Quirion R (1988). Differential alteration of various cholinergic markers in cortical and subcortical regions of human brain in Alzheimer's disease. *J Neurochem* **50**: 1914–1923.

Arnsten AF, Cai JX, Murphy BL, Goldman-Rakic PS (1994). Dopamine D1 receptor mechanisms in the cognitive performance of young adult and aged monkeys. *Psychopharmacology (Berl)* **116**: 143–151.

Auld DS, Kornecook TJ, Bastianetto S, Quirion R (2002). Alzheimer's disease and the basal forebrain cholinergic system: relations to beta-amyloid peptides, cognition, and treatment strategies. *Prog Neurobiol* **68**: 209–245.

Bach ME, Barad M, Son H, Zhuo M, Lu YF, Shih R *et al* (1999). Age-related defects in spatial memory are correlated with defects in the late phase of hippocampal long-term potentiation *in vitro* and are attenuated by drugs that enhance the cAMP signaling pathway. *Proc Natl Acad Sci USA* **96**: 5280–5285.

Bergson C, Mrzljak L, Smiley JF, Pappy M, Levenson R, Goldman-Rakic PS (1995). Regional, cellular, and subcellular variations in the distribution of D1 and D5 dopamine receptors in primate brain. *J Neurosci* **15**: 7821–7836.

Clark CR, Geffen GM, Geffen LB (1987a). Catecholamines and attention. I: Animal and clinical studies. *Neurosci Biobehav Rev* **11**: 341–352.

Clark CR, Geffen GM, Geffen LB (1987b). Catecholamines and attention. II: Pharmacological studies in normal humans. *Neurosci Biobehav Rev* **11**: 353–364.

Davies P, Maloney AJ (1976). Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet* **2**: 1403.

Day JC, Fibiger HC (1994). Dopaminergic regulation of septohippocampal cholinergic neurons. *J Neurochem* **63**: 2086–2092.

Day JC, Kornecook TJ, Quirion R (2001). Application of *in vivo* microdialysis to the study of cholinergic systems. *Methods* **23**: 21–39.

Dearry A, Gingrich JA, Falardeau P, Fremeau Jr RT, Bates MD, Caron MG (1990). Molecular cloning and expression of the gene for a human D1 dopamine receptor. *Nature* **347**: 72–76.

Demchyshyn LL, McConkey F, Niznik HB (2000). Dopamine D5 receptor agonist high affinity and constitutive activity profile conferred by carboxyl-terminal tail sequence. *J Biol Chem* **275**: 23446–23455.

Everitt BJ, Robbins TJ (1997). Central cholinergic systems and cognition. *Ann Rev Psychol* **48**: 649–684.

Flores G, Wood GK, Liang JJ, Quirion R, Srivastava LK (1996). Enhanced amphetamine sensitivity and increased expression of dopamine D2 receptors in postpubertal rats after neonatal excitotoxic lesions of the medial prefrontal cortex. *J Neurosci* **16**: 7366–7375.

Franklin KB, Paxinos G (1997). *The Mouse Brain in Stereotaxic Coordinates*. New York Academic Press: New York.

Gasbarri A, Verney C, Innocenzi R, Campana E, Pacitti C (1994). Mesolimbic dopaminergic neurons innervating the hippocampal formation in the rat: a combined retrograde tracing and immunohistochemical study. *Brain Res* **668**: 71–79.

Hersi AI, Kitaichi K, Srivastava LK, Gaudreau P, Quirion R (2000). Dopamine D-5 receptor modulates hippocampal acetylcholine release. *Brain Res Mol Brain Res* **76**: 336–340.

Hersi AI, Richard JW, Gaudreau P, Quirion R (1995a). Local modulation of hippocampal acetylcholine release by dopamine D1 receptors: a combined receptor autoradiography and *in vivo* dialysis study. *J Neurosci* **15**: 7150–7157.

Hersi AI, Rowe W, Gaudreau P, Quirion R (1995b). Dopamine D1 receptor ligands modulate cognitive performance and hippocampal acetylcholine release in memory-impaired aged rats. *Neuroscience* **69**: 1067–1074.

Hollon TR, Bek MJ, Lachowicz JE, Ariano MA, Mezey E, Ramachandran R *et al* (2002). Mice lacking D5 dopamine receptors have increased sympathetic tone and are hypertensive. *J Neurosci* **22**: 10801–10810.

Holmes A, Hollon TR, Gleason TC, Liu Z, Dreiling J, Sibley DR *et al* (2001). Behavioral characterization of dopamine D5 receptor null mutant mice. *Behav Neurosci* **115**: 1129–1144.

Imperato A, Obinu MC, Gessa GL (1993). Stimulation of both dopamine D1 and D2 receptors facilitates *in vivo* acetylcholine release in the hippocampus. *Brain Res* **618**: 341–345.

Justice Jr JB (1993). Quantitative microdialysis of neurotransmitters. *J Neurosci Methods* **48**: 263–276.

Kitaichi K, Day JC, Quirion R (1999). A novel muscarinic M(4) receptor antagonist provides further evidence of an autoreceptor role for the muscarinic M(2) receptor sub-type. *Eur J Pharmacol* **383**: 53–56.

Lapchak PA, Araujo DM, Quirion R, Collier B (1989). Binding sites for [3H]AF-DX 116 and effect of AF-DX 116 on endogenous acetylcholine release from rat brain slices. *Brain Res* **496**: 285–294.

Levin ED, McGurk SR, Rose JE, Butcher LL (1990). Cholinergic-dopaminergic interactions in cognitive performance. *Behav Neural Biol* **54**: 271–299.

Mesulam MM, Mufson EJ, Wainer BH, Levey AI (1983). Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1–Ch6). *Neuroscience* **10**: 1185–1201.

Nieoullon A (2002). Dopamine and the regulation of cognition and attention. *Prog Neurobiol* **67**: 53–83.

Packard MG, White NM (1991). Dissociation of hippocampus and caudate nucleus memory systems by posttraining intracerebral injection of dopamine agonists. *Behav Neurosci* **105**: 295–306.

Perry E, Walker M, Grace J, Perry R (1999). Acetylcholine in mind: a neurotransmitter correlate of consciousness? *Trends Neurosci* **22**: 273–280.

Perry EK, Johnson M, Kerwin JM, Piggott MA, Court JA, Shaw PJ *et al* (1992). Convergent cholinergic activities in aging and Alzheimer's disease. *Neurobiol Aging* **13**: 393–400.

Quirion R, Aubert I, Lapchak PA, Schaum RP, Teolis S, Gauthier S *et al* (1989). Muscarinic receptor subtypes in human neurodegenerative disorders: focus on Alzheimer's disease. *Trends Pharmacol Sci* **Suppl**: 80–84.

Quirion R, Hammer Jr RP, Herkenham M, Pert CB (1981). Phencyclidine (angel dust)/sigma 'opiate' receptor: visualization by tritium-sensitive film. *Proc Natl Acad Sci USA* **78**: 5881–5885.

Schultz W, Apicella P, Ljungberg T (1993). Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. *J Neurosci* **13**: 900–913.

Seeman P, Van Tol HH (1993). Dopamine receptor pharmacology. *Curr Opin Neurol Neurosurg* **6**: 602–608.

Steele TD, Hodges Jr DB, Levesque TR, Locke KW (1997). D1 agonist dihydrexidine releases acetylcholine and improves cognitive performance in rats. *Pharmacol Biochem Behav* **58**: 477–483.

Steele TD, Hodges Jr DB, Levesque TR, Locke KW, Sandage Jr BW (1996). The D1 agonist dihydrexidine releases acetylcholine and improves cognition in rats. *Ann N Y Acad Sci* **777**: 427–430.

Sunahara RK, Guan HC, O'Dowd BF, Seeman P, Laurier LG, Ng G *et al* (1991). Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. *Nature* **350**: 614–619.

Sunahara RK, Niznik HB, Weiner DM, Stormann TM, Brann MR, Kennedy JL *et al* (1990). Human dopamine D1 receptor encoded by an intronless gene on chromosome 5. *Nature* **347**: 80–83.

Tiberi M, Caron MG (1994). High agonist-independent activity is a distinguishing feature of the dopamine D1B receptor subtype. *J Biol Chem* **269**: 27925–27931.

Tiberi M, Jarvie KR, Silvia C, Falardeau P, Gingrich JA, Godinot N *et al* (1991). Cloning, molecular characterization, and chromosomal assignment of a gene encoding a second D1 dopamine receptor subtype: differential expression pattern in rat brain compared with the D1A receptor. *Proc Natl Acad Sci USA* **88**: 7491–7495.

Vaucher E, Fluit P, Chishti MA, Westaway D, Mount HT, Kar S (2002). Object recognition memory and cholinergic parameters in mice expressing human presenilin 1 transgenes. *Exp Neurol* **175**: 398–406.

Verney C, Baulac M, Berger B, Alvarez C, Vigny A, Helle KB (1985). Morphological evidence for a dopaminergic terminal field in the hippocampal formation of young and adult rat. *Neuroscience* **14**: 1039–1052.

Woolf NJ (1991). Cholinergic systems in mammalian brain and spinal cord. *Prog Neurobiol* **37**: 475–524.

Zhou QY, Grandy DK, Thambi L, Kushner JA, Van Tol HH, Cone R *et al* (1990). Cloning and expression of human and rat D1 dopamine receptors. *Nature* **347**: 76–80.