Local Modulation of Hippocampal Acetylcholine Release by Dopamine D1 Receptors: A Combined Receptor Autoradiography and *in vivo* Dialysis Study

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The modulation of *in vivo* hippocampal ACh release by dopaminergic D1 and D2 receptors was examined in this study. Additionally, in an attempt to ascertain the location of these receptors in relation to hippocampal cholinergic terminals, fimbriaectomy and quantitative autoradiography were used. Following unilateral fimbriaectomy, whereby at least 50% of hippocampal cholineacetyltransferase (ChAT) activity was lost, a significant ipsilateral decrease in D1/³H SCH23390 binding was observed in the molecular layer of the dentate gyrus while hippocampal D2/³H raclopride binding was unaffected. The effects of prototypical D1 and D2 receptor agonists and antagonists on hippocampal ACh release were examined next using *in vivo* dialysis in freely moving rats. The D1 agonist SKF 38393 (10 μ M to 100 μ M) administered directly into the hippocampus via the dialysis probe stimulated ACh release in a concentration dependent manner. The effect of the agonist was blocked by the coadministration of the D1 receptor antagonist SCH 23390 (1 μ M), which by itself failed to modulate ACh release. In contrast, neither the D2 agonist quinpirole (1–10 μ M) nor the D2 antagonist sulpiride (1–10 μ M) had any direct effect on hippocampal ACh release. Additionally, the infusion of these D1 and D2 drugs in the septal area failed to affect hippocampal ACh release. Taken together, these results suggest that a proportion of hippocampal D1 receptors are located on cholinergic nerve terminals and that dopamine, acting via D1 receptors, can locally stimulate hippocampal ACh release.

[Key words: ACh, dopamine, hippocampus, *in vivo* dialysis, receptor autoradiography, D1 receptor]

Dopamine (DA) is thought to modulate the activity of the septo-hippocampal cholinergic pathway (Robinson et al., 1979; Costa et al., 1983). Infusions of dopamine antagonists into the septum increase the firing rate of this pathway (Robinson et al., 1979) and elevate ACh turnover (Robinson et al., 1979) and high affinity choline uptake (Durkin et al., 1986) in the hippocampus.

Similar effects are also observed following either intraseptal 6-hydroxydopamine injection (Robinson et al., 1979) or the destruction of the ventral tegmental A10 dopaminergic neurons (Robinson et al., 1979; Galey et al., 1985). It has been suggested that A10 dopaminergic neurons projecting to the lateral septum interact with cholinergic fibers originating from the medial septal nucleus, possibly via septal GABAergic interneurons, to bring about this inhibitory influence (Wood, 1985). On the other hand, a variety of wide ranging studies including morphological (Scatton et al., 1980; Verney et al., 1985; Simon et al., 1989), electrophysiological (Gribkoff et al., 1984; Stanzione et al., 1984; Smialowski et al., 1987) and biochemical (Bischoff, 1979; Ishikawa, 1982) approaches provide evidence suggesting that the hippocampus receives direct dopaminergic innervation. This innervation apparently originates mainly from the ventral tegmental area (VTA; and to a lesser extent from the substantia nigra) and ascends in the medial forebrain bundle to innervate various limbic structures, including the hippocampus (Verney et al., 1985).

Dopamine receptors have been classified in two broad families (D1-like and D2-like) on the basis of the activity of various agonists and antagonists (Kebabian and Calne, 1979; Seeman, 1980). More recently, molecular cloning techniques have shown that the D1 family comprises two receptors (d1 and d5) while the D2 family consists of at least three different receptor proteins (d2, d3, and d4) (for a recent review, see Niznik and Van Tol, 1992). D1 receptors activate adenylate cyclase whereas members of the D2 receptor class have been shown to couple to numerous effector systems, including the inhibition of adenylate cyclase and the activation of potassium channels, among others (Monsma et al., 1990; Sibley and Monsma, 1992). Both the D1 and the D2 receptor subtypes have been localized in the septum as well as the hippocampus of various mammalian species including the rat (Bischoff et al., 1980; Bruink et al., 1986; Dawson et al., 1986; Grilli et al., 1988; Tiberi et al., 1991; Mengod et al., 1992).

Given this background, it is thus likely that dopamine may act as a modulator of the septo-hippocampal cholinergic pathway both at the level of the cell bodies in the septal area and at the nerve terminals within the hippocampus. In the present study, these two possibilities were investigated directly by examining the effects of local administration of selective dopamine D1 and D2 receptor agonists and antagonists on hippocampal ACh release using *in vivo* dialysis in freely moving rats as well as by evaluating the effects of unilateral fimbriaectomy on these

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receptors in the hippocampus as monitored by quantitative autoradiography. Our results suggest that a certain proportion of pharmacologically defined D1 receptors are located on cholinergic nerve terminals and that dopamine may act, via D1 receptors, as a stimulant of ACh release at level of the cholinergic nerve terminals within the hippocampus.

Materials and Methods

Materials. Male Sprague-Dawley rats (250–350 gm) obtained from Charles River Canada (St. Constant, Quebec, Canada) were maintained on a 12 hr light–dark cycle (light on at 7:00 A.M.) in temperature and humidity controlled rooms for at least 3–4 d prior to surgery. Animals were fed standard laboratory chow and had access to tap water *ad libitum*. Animal care was according to protocols and guidelines approved by the McGill University Animal Care Committee and the Canadian Council for Animal Care (CCAC).

The dialysis probes were made from AN69 Hosal fibers (molecular weight cut off < 60,000 i.d. = 220 μ m, o.d. = 310 μ m). SCH23390 HCl, (+)SKF38393 HCl, (–)SKF 38393 HCl, quinpirole HCl, sulpiride, and eticlopride HCl were obtained from RBI (Waukegan, MA). Neostigmine Bromide was purchased from BAS (West Lafayette, IN). 3 H SCH23390 (80.7 Ci/mmol), 3 H raclopride (70.0 Ci/mmol), 3 H-Hyperfilms and microscale standards were purchased from Amersham Canada (Oakville, Ontario, Canada). The deuterated variant of ACh, 3 H₂ ACh bromide [(CH₃)₃NBrCD₂CD₂OC(=O)CH₃], used as internal standard for ACh determination was obtained from Merck, Sharp and Dohme Isotopes (Montreal, Quebec, Canada). Developer (D-19) and fixer (Rapid Fix) were obtained from Kodak Chemical Inc. (Montreal, Quebec, Canada). All other reagents and chemicals were of HPLC or GC-MS grade and purchased from either Fisher Scientific Co. (Montreal, Quebec, Canada) or Aldrich Chemicals (Chicago, IL).

Fimbriaectomy. The fimbria fornix of male rats was unilaterally interrupted by a knife cut lesion under sodium nembutal anesthesia (50 mg/kg). Briefly, at a 90° angle and coordinates of 1 mm behind bregma and 3.0 mm lateral to the midline suture (Paxinos and Watson, 1982), a leukotome knife (Kopf Instruments) was lowered via an opening in the skull to a depth of 4.0 mm below dura. The wire in the knife was then extended under the fimbria and the leukotome slowly brought back to the dura. For sham operated animals, the leukotome was lowered and brought back to the surface as above but without extending the wire. The animals were allowed to recover from anesthesia under a warm light and individually housed according to CCAC guidelines. Two weeks postsurgery, the animals were sacrificed and the efficacy of the lesions was assessed by determining hippocampal ChAT activity.

Hippocampal ChAT activity. Hippocampal punches from 300 μ m brain slices of the lesioned and sham operated animals were assayed for ChAT activity. Homogenates from these punches were incubated for 15 min in a buffer containing 14 C acetyl CoA as previously described in detail (Araujo et al., 1988) using the method of Fonnum (1969) as modified by Tucek (1978). The animals that were used in subsequent receptor autoradiographic studies showed hippocampal ChAT activity losses of $51 \pm 4\%$ on the lesioned side as compared to the contralateral hippocampus.

Dopamine receptor autoradiography. The status of hippocampal dopaminergic receptors following fimbriaectomy was assessed as described in detail elsewhere (Debonnel et al., 1990). In brief, following sectioning at -17°C , 20 μ m hippocampal slices were incubated for 60 min at room temperature in 50 mM Tris HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, and 1.0 nM 3 H SCH 23390 for D1 receptors or 5.7 nM 3 H raclopride for D2 receptors. Serial sections were incubated in this buffer but with the addition of 1 μ M SCH 23390 or 1 μ M (+) butaclamol to ascertain the specificity of the D1 or D2 radioligand binding, respectively. The sections were then rinsed five times (2 min each) in fresh ice-cold buffer. Buffer salts were removed by a rapid dip in ice-cold distilled water and the sections rapidly air dried. Autoradiograms were generated by apposing the sections alongside with tritium standards to tritium sensitive films for 4 weeks. The films were then developed as described before (Quirion et al., 1981) and 3 H SCH 23390 and 3 H raclopride binding quantified (fmol/mg tissue wet weight) using computer assisted microdensitometric image analysis system (MCID System, Imaging Research Inc., St. Catherine's, Ontario, Canada). Although, the D1 radioligand, 3 H SCH 23390, also binds 5HT₂ receptors (Bischoff et al., 1986), under the conditions used in this study, this ligand is likely preferentially labeling

D1 receptors (Dawson et al., 1986). Anatomical areas were identified according to the Paxinos and Watson's atlas (1982). Significant differences between experimental groups ($n = 4$ in each group) were determined by a one-way analysis of variance (ANOVA).

Probe implantation and hippocampal *in vivo* dialysis. Male Sprague-Dawley rats (250–350 gm) were anesthetized with sodium nembutal (50 mg/kg). Transverse probes (Damsma et al., 1987) were stereotactically implanted in the dorsal hippocampus and for septally manipulated animals also in the lateral septum (Giovannini et al., 1994) at coordinates of 3.8 mm posterior to bregma and 3.5 mm below the skull for the hippocampus and 0.7 mm anterior to bregma and 4.5 mm below the skull for the septum (Paxinos and Watson, 1982). The animals were individually housed and allowed to recover from surgery for 2 d prior to their use in the *in vivo* dialysis experiments. Each animal was dialyzed only once.

At the beginning of each dialysis experiment, animals were placed in lidless cages and connected to a BAS microliter syringe pump in a manner as to allow them to freely move in the cages. The probes were perfused for a one hour wash out period at a flow rate of 2.34 μ l/min with an Ungerstedt-Ringer solution (127 mM NaCl, 2.5 mM KCl, 1.0 mM MgCl₂, 1.3 mM CaCl₂, pH 7.4) containing 5 μ M neostigmine bromide, a cholinesterase inhibitor. Twenty five minute dialysate fractions were collected into a 1 ml glass vials containing 46 μ l of 0.1 N HCl and 50 pmol of deuterated ACh as internal standard. Following about 3 hr of baseline hippocampal ACh release, drugs of interest were tested by inclusion in the perfusion Ringer's solution for the remainder of the dialysis experiment. For septally manipulated animals, the drugs of interest were perfused into the septum in a Ringer's solution lacking neostigmine. The samples were frozen immediately and stored at -80°C until assayed by gas chromatography/mass spectroscopy (GC/MS). Following most experiments, probe location was verified by standard histological examination of the brain.

In the experiment designed to evaluate the potential diffusion of the locally applied drugs, the *in vivo* dialysis setup was as described above for hippocampally manipulated animals except that 3 H SCH 23390 (1 μ M) was used and no samples were collected. The animals were sacrificed immediately following the termination of the experiment. Twenty micrometer slices of the brains of these animals were exposed to tritium sensitive Hyperfilms for 10 d.

GC/MS analysis of ACh. ACh content of the dialysate fractions was determined by GC-MS as described in detail by Marien and Richard (1990). Briefly, frozen samples were lyophilized overnight, reconstituted in 250 μ l acetonitrile, capped, heated at 80°C for 30 min, and dried under a gentle stream of nitrogen gas. Quaternary amines present in the samples were demethylated by adding 250 μ l sodium benzene thiolate solution (160 mg in 18 ml of redistilled methyl ethyl ketone and 35 μ l glacial acetic acid) under a flow of nitrogen and reacting at 80°C for 45 min. Samples were then extracted into 35 μ l citric acid and washed twice with 250 μ l pentane. Finally the samples were extracted into 80 μ l of ethyl acetate and concentrated down to 3–5 μ l volume before being injected in to the GC-MS (Hewlett-Packard 5987b). The demethylated derivatives of ACh were analyzed by selective ion monitoring of 132 atomic mass units (amu) for endogenous and 136 amu for the internal standard, 3 H₂-ACh.

The amount of endogenous ACh in each dialysate was calculated (Jenden and Hanin, 1974) from the peak area ratio of endogenous versus deuterated internal standard. Calculations were not corrected for the recovery of ACh by each dialysis probe. Sample ACh content was expressed as a percentage of average baseline (eight sample collections preceding drug infusion). Significant differences between experimental groups were determined by a one-way analysis of variance (ANOVA).

Results

Effect of fimbriaectomy on hippocampal D1 and D2 receptor binding sites

A significant loss (18.2%, $p = 0.0038$) of D1/ 3 H SCH23390 binding was observed in the molecular layer of the dentate gyrus of the ipsilateral hippocampus in lesioned animals (29.9 ± 0.8 vs 36.6 ± 1.1 fmol/mg tissue wet weight) (Fig. 1A,B). No detectable differences were observed in 3 H SCH 23390 binding in CA1, CA2, and CA3 subfields between the ipsi- and contralateral hippocampi of fimbriaectomized rats (Fig. 1A,B). 3 H SCH 23390 binding levels were similar in the hippocampus of sham

Figure 1. Effect of fimbriaectomy on hippocampal D1/ ^3H SCH 23390 and D2/ ^3H raclopride binding. Data represent mean $\pm$ SEM from four different animals expressed in fmol/mg tissue, wet weight. Sections from unilaterally fimbriaectomized rats were incubated with 1.0 nM ^3H SCH 23390/D1 (A, B) or ^3H raclopride/D2 (C, D) as described in Materials and Methods. Nonspecific binding was defined in the presence of 1 μM SCH 23390 or 1 μM (+) butaclamol for D1 and D2, respectively. Autoradiograms (A, C) generated by apposing the sections against tritium sensitive films were subsequently quantified (B, D) using computer assisted image analysis system. The histograms represent specific labeling obtained by subtracting nonspecific from total binding. Solid and shaded bars represent the ipsi- and contralateral, respectively. Statistical analysis was evaluated by one-way analysis of variance (ANOVA). *, $p < 0.05$; significantly different from nonlesioned contralateral side. The bar equals 2 cm. Abbreviations: CA, Ammon's horn; CPu, caudate-putamen; DG, dentate gyrus; Sub, subiculum.

operated animals as compared to the contralateral hippocampi of the fimbriaectomized animals (data not shown).

Hippocampal D2/³H raclopride binding was considerably lower than that of D1/³H SCH 23390. Using ³H raclopride, the only detectable amount of specific binding in the dorsal hippocampus was observed in the CA1 and subicular regions (Fig. 1C). No differences were seen between ipsi- and contra-lateral hippocampi of unilaterally fimbriaectomized animals (Fig. 1D). Additionally, no detectable changes of either ³H SCH 23390 or ³H raclopride binding levels were observed in the septum or cortical regions as a result of unilateral fimbriaectomy. In all the animals used here, ChAT activity in the ipsilateral hippocampi decreased by $51 \pm 4\%$ as compared to the contralateral hippocampi or the hippocampus of sham operated animals.

Local dopaminergic receptor modulation of hippocampal ACh release

The average basal efflux of ACh from the dorsal hippocampus was 4.8 pmol/25 min ($n = 52$). Moreover, there were no appreciable differences in basal ACh release between the various experimental groups studied here. Hippocampal intraprobe administration of the active enantiomer of the selective D1 agonist (+)SKF 38393 (10–100 μ M), but not the inactive enantiomer (–)SKF 38393 (10 μ M), increased ACh release in a concentration dependent manner (Fig. 2A). The D1 antagonist SCH 23390 (1–10 μ M), by itself, had no appreciable effect on hippocampal ACh release in freely behaving animals (Fig. 2B). However, the coinfusion of the D1 antagonist (1 μ M) with the D1 agonist (10 μ M) blocked the stimulatory effect of the latter (Fig. 2C).

Under the conditions used here, neither the D2 agonist quinpirole HCl (1–10 μ M) (Fig. 3A) nor the D2 antagonists sulpiride (1–10 μ M) (Fig. 3B) or eticlopride (10 μ M, data not shown) significantly modified hippocampal ACh release. Interestingly, when infused in the lateral septum neither the D1 nor D2 drugs had an effect on hippocampal ACh release (Fig. 4). No appreciable obvious behavioral sequelae (locomotion, sniffing, grooming, etc.) were induced by these dopaminergic drugs administered via the probe.

Diffusion of hippocampally infused ³H SCH23390

³H SCH 23390 (1 μ M, 1 μ Ci) infused via the probe for 4 hr showed virtually no diffusion beyond the hippocampal area immediately in contact with the dialysis probe (Fig. 5). Considering that this concentration of the unlabelled D1 antagonist blocked the stimulation of hippocampal ACh release induced by the D1 agonist SKF 38393, it suggests that this effect is occurring locally.

Discussion

A variety of studies have demonstrated the presence of direct dopaminergic innervation into the hippocampus. This innervation is concentrated in the ventral subiculum, in particular in the presubiculum (Verney et al., 1985). Hippocampal D1 receptors, on the other hand, are mainly located in the molecular layer of the dentate gyrus and the dorsal hippocampus (Dawson et al., 1986; Grilli et al., 1988; Tiberi et al., 1991). In the present study, we observed that a significant portion of D1 receptors in the molecular layer of the dentate gyrus was lost following fimbriaectomy. This suggests that a proportion of hippocampal D1 receptors are located presynaptically on afferent terminals. Moreover, a concomitant reduction in hippocampal ChAT activity was observed as a result of the lesion. Considering that a

Figure 2. Effects of the infusion of the D1 agonist (+)SKF 38393 [A: 10 μ M, $\square$; 100 μ M, $\blacksquare$; or its inactive enantiomer (–)SKF 38393 10 μ M, $\triangle$], the D1 antagonist SCH 23390 [B: 1 μ M, $\square$; 10 μ M, $\blacksquare$], or their combination [C: 10 μ M (+)SKF 38393 and 1 μ M SCH 23390] on hippocampal ACh efflux. Data represent mean $\pm$ SEM ($n = 4$ for each concentration). Following baseline determination, Ringer's solution containing the drug of interest was perfused through the probe implanted in the hippocampus for the period indicated by the black bars (upto 4 hr). Dialysate ACh content, measured by GC-MS, is expressed as a percent of baseline. Baseline was calculated from the average of eight samples preceding drug infusion. Statistical evaluation was evaluated by one-way analysis of variance (ANOVA). *, $p < 0.001$; significantly different from baseline. Only the D1 agonist induced significant effects (A) that were blocked by the a coinfusion of the antagonist (C).

Figure 3. Effects of the infusion of the D2 agonist quinpirole (*A*: 1 μM , $\square$; 10 μM , $\blacksquare$) and the D2 antagonist sulpiride (*B*: 1 μM , $\square$; 10 μM , $\blacksquare$) on hippocampal ACh efflux. Data represent mean $\pm$ SEM ($n = 4$ for each concentration). Following baseline determinations, Ringer's solution containing the drug of interest was perfused through the probe implanted in the hippocampus for the period indicated by the solid bars (up to 4 hr). Dialysate ACh content, measured by GC-MS, is expressed as a percent of baseline. Baseline was calculated from the average of eight samples preceding drug infusion.

et al., 1985), it is logical to assume that at least some of the lost D1 receptors are located on cholinergic nerve terminals. Interestingly, in senile dementia of the Alzheimer's type (SDAT), a marked reduction in hippocampal D1 receptors has been reported (Cortes et al., 1988), with the highest loss (89%) seen in the molecular layer of the dentate gyrus. It is well known that one of the hallmarks of SDAT is the destruction of basal forebrain cholinergic neurons which, in part, project to the hippocampus (Davies and Maloney, 1976; Whitehouse et al., 1982; Coyle et al., 1983). Therefore, in agreement with these studies, the re-

Figure 4. Effects of the septal infusion of the D1 ligands (*A*: SKF 38393 10 μM , $\square$; SCH 23390 10 μM , $\blacksquare$) and the D2 ligands (*B*: quinpirole 10 μM , $\square$; sulpiride 10 μM , $\blacksquare$) on hippocampal ACh efflux. Data represent mean $\pm$ SEM ($n = 4$ for each ligand). Following baseline determinations, Ringer's (minus neostigmine) solution containing the drug of interest was perfused through the probe implanted in the septum for the period indicated by the black bars (up to 4 hr). Samples were collected from the probe implanted in the dorsal hippocampus. Dialysate ACh content, measured by GC-MS, is expressed as a percent of baseline. Baseline was calculated from the average of eight samples preceding drug infusion.

duction in hippocampal D1 receptors observed here could be due to the loss of presynaptically located receptors resulting from the cholinergic denervation. Nevertheless, the possibility that some of these D1 receptors are located on afferents other than cholinergic that are contained within the fimbria-fornix projection cannot be excluded solely on the basis of the present experiments.

D2 receptors, on the other hand, are found in the hippocampus

Figure 5. Diffusion of trans-hippocampally infused ^3H SCH 23390 in brain parenchyma. ^3H SCH23390 (1 μM , 1 mCi/mmol) was perfused for 4 hr in the dorsal hippocampus as described in Materials and Methods; 20 μm brain sections from these animals were exposed to tritium sensitive films and photomicrographs taken. **A** shows the extent of diffusion of the radioactive material; **B**, a schematic representation of the section in **A**. The arrows mark the location of the dialysis probe. It is rather evident that diffusion was minimal and the infused radioactivity remained in close proximity to the dialysis probe. Abbreviations: CA, Ammon's horn; DG, dentate gyrus; HY, hypothalamus; TH, thalamus.

in very low amounts compared to D1 receptor levels (Fig. 1D). In the dorsal hippocampus, D2/ ^3H raclopride binding sites are mainly located in the CA1 and subiculum regions (Fig. 1C). In the present study, no alteration in hippocampal D2 receptor densities were observed following fimbriaectomy. It would thus appear that this dopamine receptor subtype is not directly associated with the septo-hippocampal cholinergic nerve projection.

Recently, the technique of *in vivo* dialysis has been used to study the possible regulation by dopaminergic drugs of the septo-hippocampal cholinergic pathway. Systemic administration of dopaminergic drugs has shown that dopamine potentially stimulates hippocampal ACh release via both D1 and D2 receptor subtypes (Imperato et al., 1993) or the D1 subtype alone (Day and Fibiger, 1994) in young animals, and at least via D1 receptors in aged-memory impaired rats (Hersi et al., 1994). A number of possible loci exist for this apparent interaction between DA and ACh. Given the direct dopaminergic innervation of the hippocampus and the putative localization of D1 receptors on hippocampal terminals (see above), a direct modulation of hippocampal ACh release by DA drugs is a likely possibility.

The D1 receptor agonist (+)SKF 38393 applied directly into the hippocampus via the dialysis probe stimulated, in a concentration dependent, ACh release. This effect was blocked by the D1 antagonist SCH 23390 while the inactive stereoisomer (–)SKF 38393 had no effect by itself, attesting to the specificity of the effect for the D1 receptor family. SCH 23390 infused alone in the dialysis probe did not alter hippocampal ACh levels. These findings suggest that the D1 receptors located in the dorsal hippocampus can enhance ACh release and that this action is likely phasic in nature, in view of the lack of effect of the D1 antagonist alone.

A question arises, however, as to the extent of diffusion of locally applied drugs in the *in vivo* dialysis paradigm used here. This is especially pertinent considering the relatively long period of drug infusion. Administration of ^3H SCH 23390 under conditions identical to those used for the nonradioactive drugs, showed that diffusion is rather minimal and that the ligand is mostly, if not exclusively, found in the area surrounding the dialysis probe (Fig. 5). Thus, it would appear that local interaction can account for the effects of D1 drugs on hippocampal ACh release as observed in the present study or following systemic administration (Imperato et al., 1993; Day and Fibiger, 1994; Hersi et al., 1994).

In contrast to D1 drugs, neither the stimulation nor the blockade of D2 receptors by local, intraprobe infusion of prototypical

drugs into the dorsal hippocampus had any effect on ACh release.

Another possible locus for ACh/DA interaction is the lateral septal area. Dopaminergic projections arising from the VTA and terminating in the lateral septum were postulated to interact with the cholinergic cell bodies of the medial septum that give rise to the cholinergic septo-hippocampal pathway (Wood, 1985). In the present study, the manipulation of neither D1 nor D2 receptors in the septal area had any effect on hippocampal ACh release. Thus, it would appear that dopamine stimulates hippocampal ACh release by acting on D1 receptors located in the hippocampus. However, although the lesion data presented here suggests that these D1 receptors are located on cholinergic terminals, the possibility of transsynaptic action involving interneurons or other noncholinergic afferents cannot be ruled out at the present time.

In the classical view of the synapse, a close juxtaposition must exist between the nerve fiber terminals enriched with a given neurotransmitter and its postsynaptic receptors. However, this is clearly not always the case and may even be the exception (Beaudet and Descarries, 1978). Discrepancies between the localization of receptors and the distribution of the relevant neurotransmitter has been referred to as the mismatch issue (Kuhar, 1985; Herkenham, 1987). In the present situation, the hippocampal dopaminergic innervation, arising mainly from the VTA and the substantia nigra, is mostly restricted to the ventral hippocampus (Verney et al., 1985). In contrast, hippocampal dopaminergic receptors are predominantly found in the dorsal hippocampus (Dawson et al., 1986; Grilli et al., 1988; Tiberi et al., 1991; this study). Interestingly, however, more than 40% of the dopamine present in the hippocampus has been proposed to be located within dorsal noradrenergic terminals (Bischoff et al., 1979). It may be conceivable that, under certain conditions, dopamine and noradrenaline may be coreleased from the terminals of the latter present in the dorsal hippocampus. It is also tempting to suggest that crossover between neurotransmitters may exist to partly account for the apparent mismatches. For instance, under excessive cell firing conditions, the release of noradrenaline in quantities large enough to saturate its own receptors may lead to the binding of this neurotransmitter to other related receptor families such as those for dopamine. In this way, a secondary level of complexity would be added to code for signal strength and more effective transmission.

Another intriguing possibility to account for apparent ligand–receptor mismatches is known as volume transmission (VT). In

contrast to the classical synaptic mode of signal transmission, VT refers to the diffusion of chemical signals in the extracellular fluid. A neurotransmitter could thus act at a considerable distance from its site of release (for a recent review, see Benfenati and Agnati, 1991). For example, it has been reported that when transient parkinsonism is induced in cats following MPTP administration, dopamine released from spared ventral striatum terminals can diffuse over a distance of 5.5–7.0 mm to the more extensively denervated dorsolateral striatum (Schneider et al., 1994). Similarly, dopamine released from fiber terminals located in the ventral hippocampus could diffuse to the dorsal part where the dopaminergic D1 receptors are mostly found. However, direct evidence in support of this possibility have yet to be provided. Interestingly, D1 receptors have, in the past, been linked with volume transmission in areas such as the median eminence and globus pallidus (Fuxe et al., 1988). In any case, it is evident from the data reported here that the activation of D1 receptors present in the dorsal hippocampus can modulate ACh release and are hence fully functional.

Finally, it should be added that the physiological significance of the dopaminergic system in the hippocampus is not yet clear. Intrahippocampal injections of dopamine receptor agonists evoke theta rhythms in hippocampal electroencephalograms (Smialowski, 1985). This type of rhythmic oscillation is known to occur during periods of learning (Winson, 1978). Moreover, during theta oscillation, hippocampal synapses are in a state of heightened plasticity and the stimulatory requirements for the induction of long term potentiation (LTP) are markedly reduced (Huerta and Lisman, 1993). Interestingly, a well established role of the hippocampal cholinergic nerve terminals is to elicit theta rhythms (Bland, 1986). Most recently, dopamine was reported to modify LTP in the Schaffer collateral pathway of the rat hippocampus via D1 receptors (Huang and Kandel, 1995). In addition, mnemonic deficits in aged rats can be attenuated by D1 receptor agonists, and this effect was proposed to be mediated by the release of ACh in the hippocampus (Hersi et al., 1994). Therefore, it appears that a role for DA in the hippocampus could, at least in part, be associated with learning and memory, likely via the modulation of hippocampal cholinergic functions.

In summary, a certain proportion of hippocampal D1 receptors appears to be located directly on septo-hippocampal cholinergic nerve terminals. Moreover, *in vivo* hippocampal ACh release is facilitated by the local stimulation of D1 but not D2 receptors. Recent data have clearly shown that the D1 receptor family comprises both the d1 and d5 subtypes (see introductory section). At present, it is unclear which of these two subtypes is involved in the modulation of hippocampal ACh release as selective probes have yet to be developed to discriminate between these members of the D1 receptor family. Other strategies such as the use of functional receptor antibodies or oligonucleotide antisenses would have to be used.

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