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# GABA receptors and benzodiazepine binding sites modulate hippocampal acetylcholine release in vivo

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**Abstract**

In the present study, the regulation of acetylcholine release from the ventral hippocampus by γ-aminobutyric acid (GABA) was investigated in vivo. GABA receptor agonists and antagonists were administered locally in the medial septum and the adjacent vertical limb of the diagonal band of Broca, or in the hippocampus by retrograde dialysis. Acetylcholine release was measured in the ventral hippocampus. In addition, the modulation of acetylcholine release via septal benzodiazepine binding sites was assessed by intraseptal administration of an agonists and an antagonist at the benzodiazepine binding site. Intraseptal administration of the $GABA_A$ receptor agonist muscimol and the $GABA_B$ receptor agonist baclofen, but not the agonist of the benzodiazepine binding site midazolam, decreased acetylcholine release in the hippocampus. The $GABA_A$ receptor antagonist bicuculline and the antagonist of the benzodiazepine binding site flumazenil, but not the $GABA_B$ receptor antagonist 3-*N*-(3,4,-dichlorobenzyl) aminopropyl-*P*-diethoxymethyphosphinic acid (CGP 52432) increased acetylcholine release in the hippocampus upon intraseptal administration. The same GABA receptor ligands were administered in the ventral hippocampus. CGP 52432 induced a small increase in acetylcholine release, whereas baclofen, muscimol and bicuculline did not affect local acetylcholine release. Thus, endogenous GABA causes tonic inhibition of acetylcholine release in the ventral hippocampus via septal $GABA_A$ receptors and, to a lesser extent, via $GABA_B$ receptors in the medial septum and hippocampus. The GABAergic inhibition in the medial septum is reduced by antagonists of the benzodiazepine binding site. 

*Keywords:* Acetylcholine; Hippocampus; Septum; GABA receptor; Benzodiazepine binding site; Alzheimer's disease

## 1. Introduction

Cholinergic neurotransmission in the hippocampus and cortex is regulated by the inhibitory amino acid neurotransmitter γ-aminobutyric acid (GABA). Several lines of evidence indicate that GABA acts on acetylcholine release by inhibiting the firing of basal forebrain cholinergic neurons that project to the hippocampus and cortex.

Cholinergic neurons in the medial septum and the vertical limb of the diagonal band of Broca are the major source of cholinergic afferents to the hippocampus (Amaral and Kurz, 1985; Wainer et al., 1985; Linke et al., 1994). Cholinergic neurons in the septum express subunits of the $GABA_A$ receptor (Gao et al., 1995) and receive GABAergic innervation, probably from local GABAergic neurons and hippocampo-septal neurons (Leranth and Frotcher, 1989; Gaykema et al., 1991; Tóth et al., 1993). Injection of the $GABA_A$ receptor agonist muscimol into the medial septum reduces acetylcholine release, acetylcholine turnover rate and high-affinity choline uptake and in the hippocampus (Costa et al., 1983; Wood, 1986; Durkin, 1992; Walsh et al., 1993; Gorman et al., 1994). GABA also inhibits spontaneous firing activity in the majority of septo-hippocampal neurons (Bassant et al., 1988; Lee et al., 1991) and mediates hyperpolarisation of medial septal neurons (Schneggenburger et al., 1992; Segal, 1986). Aspects of brain activity that are related to hippocampal acetylcholine, like hippocampal slow rhythmic activity (theta-rhythm) and spatial and working memory (Allen and Crawford, 1984; Chrobak et al., 1989; Givens and Olton, 1990; Durkin, 1992; Bland et al., 1996), are also affected by administration of muscimol in the septal area. In paral-

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lel, cholinergic activity in the cortex is reduced by administration of GABA receptor agonists into the nucleus basalis/substantia innominata (Wenk, 1984; Casamenti et al., 1986; Wood, 1986).

$GABA_A$ receptors in the medial septum and the diagonal band have binding sites for benzodiazepines. Benzodiazepines are widely used as anxiolytic and anticonvulsive agents that act by augmenting $GABA_A$ receptor-mediated inhibition. Benzodiazepines also induce transient anterograde amnesia in humans and experimental animals (see Lister, 1985 and Thiebót, 1985 for reviews) and decrease acetylcholine release in the hippocampus and cortex (Imperato et al., 1993, 1994; Moore et al., 1993; Dazzi et al., 1996). Accordingly, it has been suggested that benzodiazepines cause amnesia by inhibiting the activity of cholinergic neurons in the basal forebrain. Local administration of benzodiazepines into the basal forebrain decreases acetylcholine release in the hippocampus and cortex (Imperato et al., 1993, 1994) and impairs learning and memory performance (McNamara and Skelton, 1995; Stackman and Walsh, 1995). Antagonists and inverse agonists at the benzodiazepine binding site increase cholinergic activity and acetylcholine release in the hippocampus (Imperato et al., 1993, 1994; Walsh et al., 1993) and frontal cortex (Moore et al., 1993, 1995), and enhance memory performance (Mayo et al., 1992; McNamara and Skelton, 1993; Herzog et al., 1996) when administered either systemically or into the basal forebrain. Because inverse agonist of the benzodiazepine binding site increase acetylcholine release and improve memory performance, they might be useful in the treatment of amnesia in Alzheimer's disease, where amnesia is thought to be related to a decline in cholinergic neurotransmission in the hippocampus and cortex (Sarter et al., 1990; Walsh, 1993).

Despite the overwhelming evidence for the role of GABA in the regulation of cholinergic activity in the hippocampus, a number of basic questions have not been resolved.

(1) It is unclear whether $GABA_A$ receptors mediate tonic inhibition of hippocampal acetylcholine release in vivo.

(2) The role of the $GABA_B$ receptors in the regulation of hippocampal acetylcholine release is unclear because the small number of studies on this subject have yielded conflicting results.

(3) In addition to GABA receptors in the medial septum, GABA receptors in the hippocampus may also affect local acetylcholine utilisation.

In the present study, we addressed these questions by administering $GABA_A$ and $GABA_B$ receptor agonists and antagonists locally in either the medial septum and the vertical limb of the diagonal band, or in the ventral hippocampus. In addition, the modulation of acetylcholine release via septal benzodiazepine binding sites was evaluated by intraseptal administration of an agonist and an antagonist at the benzodiazepine binding site.

## 2. Materials and methods

### 2.1. Animals and surgery

Adult male albino rats of a Wistar derived strain, weighing 280–320 g (Harlan, The Netherlands), were anaesthetised with chloral hydrate (400 mg/kg i.p.) and placed in a stereotaxic frame (Kopf, USA). Dialysis probes were implanted in the right ventral hippocampus, aiming at subfield 3 of Ammon's horn (IA, +3.7 mm; lateral, −4.8 mm; and ventral, −8.0 mm from dura mater), and in the medial septum and adjacent tip of the vertical limb of the diagonal band of Broca (IA, +9.5 mm, lateral, +1.0 mm, ventral, −7.0 mm), as previously described in Moor et al. (1994).

Home-made I-shaped probes, made of a polyacrylonitrile/sodium methallyl sulfonate copolymer dialysis tube (inner diameter: 0.22 mm, outer diameter: 0.31 mm, Hospal, Italy) were used. The exposed tips of the dialysis membranes were 2.5 mm (medial septum) and 4 mm (hippocampus). Following surgery, rats were housed individually in Plexiglas chambers (25 cm × 25 cm × 35 cm) with free access to food and water. Animal procedures were conducted in accordance with guidelines published in the National Institute for Health Guide for the Care and Use of Laboratory Animals, and all protocols were approved by the Groningen University Commission for Animal Care and Use.

### 2.2. Microdialysis experiments

Dialysis experiments were conducted during daytime, 16–20 h following surgery. The probes were perfused with artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl 147, KCl 3.0, $CaCl_2$ 1.2 and $MgCl_2$ 1.2. The aCSF was delivered by a syringe pump (CMA, Sweden) at a rate of 2 μl/min. The acetylcholinesterase inhibitor neostigmine–bromide (100 nM; Sigma, St. Louis, USA) was added to the aCSF (in the hippocampus only). Dialysate-fractions (15 min) were collected in a 50-μl loop of an injection valve (Valco, Switzerland), controlled by an electronic timer. Experiments started following stabilisation of acetylcholine levels (4 samples within ±25% variation). Drug were administered after collection of 5 basal samples. Drugs were dissolved in the aCSF and perfused continuously in the dialysis probe for 75–90 min (5–6 samples). Following the dialysis experiments, rats were killed and the placement of the probes was controlled in brain slices.

### 2.3. Chemical analysis

Samples were analysed 'on-line' using high-performance liquid chromatography, enzymatic conversion and electrochemical detection, as previously described by Damsma et al. (1987), with some minor adjustments.

Briefly, the samples were injected onto a reverse-phase $C_{18}$ column preloaded with sodium laurylsulphate. Acetylcholine was converted into hydrogen peroxide and betaine in a post-column enzyme-reactor containing immobilised acetylcholinesterase and choline-oxidase (Sigma, St. Louis, MO, USA). Subsequently, hydrogen peroxide was detected using a platinum electrode (Antec, Leiden, The Netherlands) set at +500 mV versus a Ag/AgCl reference electrode. The mobile phase was a 0.15 M potassium phosphate buffer (pH = 8.0) containing 0.5 mM ethylene–diaminetetra–acetate, 2 mM tetramethylammonium chloride (Merck, Germany), and 0.05% Kathon (Rohm and Haas, UK), and was delivered at a rate of 0.4 ml/min. Acetylcholine content was calculated by comparing the peak with that of a 5 pmol standard (50 μl of 0.1 μM acetylcholine solution) and is expressed in fmol/min. Values were not corrected for probe recovery. The stability of detection was controlled by injection of standards before and after each experiment. The detection limit of the assay was approximately 20 fmol/sample.

### 2.4. Drugs

The following drugs were used: muscimol HBr, (−)-bicuculline, (±)-baclofen and kainic acid, all purchased from RBI (Natick, MA, USA). Midazolam HCl was purchased from Hoffmann-La Roche (Basel, Switzerland), whereas flumazenil was a generous gift from the company. 3-*N*-(3,4,-dichlorobenzyl) aminopropyl-*P*-diethoxymethyphosphinic acid (CGP 52432) was kindly donated by Ciba Geigy (Basel, Switzerland). Drug concentrations were chosen according to results of previous studies (Moor et al., 1998; Santiago and Westerink, 1992) or pilot experiments. In general, drug concentrations were sufficient to elicit an effect if the relevant receptor was involved in the regulation of acetylcholine release, but low enough to prevent non-specific receptor binding or significant changes in local ion composition.

### 2.5. Data analysis and statistical methods

Acetylcholine values from each animal were transformed to percent of the average basal value in the same animal and are shown in the figures as means for a treatment group. The effects of drug administration were evaluated with a non-parametric repeated measures analysis of variance (Friedman's method) followed by a multiple comparison against the last basal sample (Dunnett's method). Significance level for all statistical procedures was set at $P < 0.05$.

## 3. Results

The average output of acetylcholine in the ventral hippocampus was 357 ± 28 fmol/sample (S.E.M., $n = 63$).

### 3.1. GABA receptor agonists and antagonists in the medial septum

Perfusion with 10–100 μM muscimol in the medial septum induced a distinct change in behaviour. This change included increased locomotor activity, rearing and sniffing (not shown). Therefore, further experiments with intraseptal muscimol were conducted in halothane-anaesthetised rats. None of the other drugs induced evident changes in behaviour. Acetylcholine release decreased to 76 ± 14% (mean ± S.E.M, $n = 6$) of baseline during anaesthesia. Muscimol administration (100 μM) in the medial septum decreased acetylcholine levels further to 59% of the levels measured in anaesthetised animals ($\chi^2(10) = 35.5$, $P < 0.001$). Acetylcholine levels remained low after muscimol administration was stopped.

Intraseptal administration of bicuculline (5 μM) increased acetylcholine release to a maximum of 187 ± 9% of baseline after 45 min ($n = 6$, Fig. 1). Statistical analysis indicated that the effect of drug treatment was significant ($\chi^2(10) = 42.9$, $P < 0.001$). Acetylcholine release returned to baseline levels 1 h after bicuculline administration.

Perfusion with the $GABA_B$ receptor agonist (±)-baclofen (50 μM) in the medial septum induced a small decrease in hippocampal acetylcholine release in the hippocampus ($\chi^2(10) = 22.9$, $P < 0.05$). Acetylcholine release was significantly lower than baseline after 60 min of drug treatment (72 ± 4%, $n = 7$, Fig. 2). Administration of the $GABA_B$ receptor antagonist CGP 52432 (30 μM, $n = 5$, Fig. 2) in the medial septum failed to affect acetylcholine release in the hippocampus ($\chi^2(10) = 4.1$, n.s.).

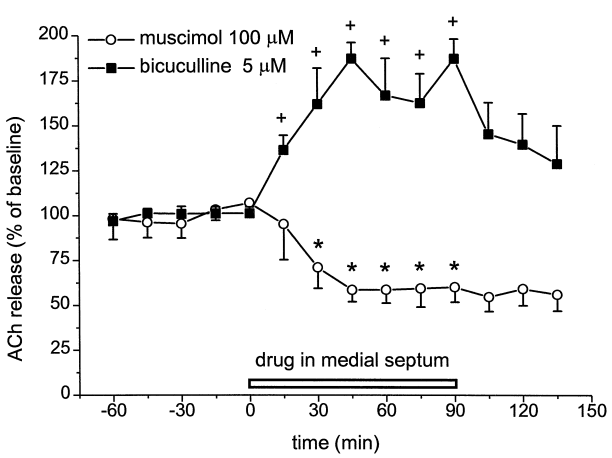


Fig. 1. Effects of administration of the $GABA_A$ receptor agonist muscimol (100 μM, $n = 6$) and antagonist (−)bicuculine (5 μM, $n = 6$) in the medial septum on acetylcholine release in the ventral hippocampus. All values are shown as percent of baseline release (5 pre-treatment samples). Drugs were dissolved in aSCF and administered by retrograde dialysis. Symbols indicate values that are significantly different from last baseline value in experiments with muscimol (*) and bicuculline (+), respectively (post-hoc Dunnett's multiple comparison procedure, $P < 0.05$). Experiments with muscimol were performed in halothane-anaesthetised rats (see text for explanation).

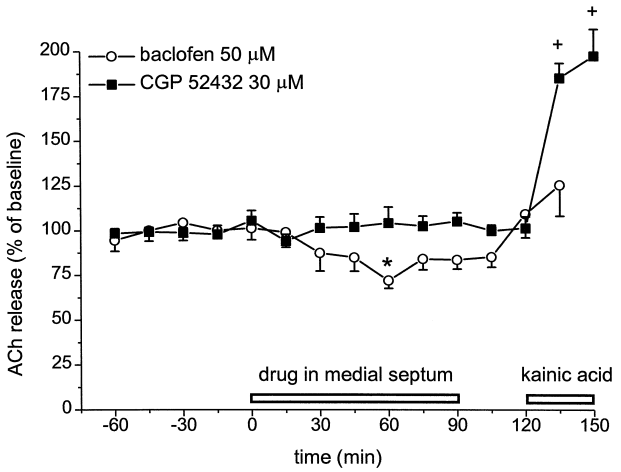


Fig. 2. Effects of the $GABA_B$ receptor agonist (±)-baclofen (50 μM, $n = 7$) and antagonist CGP 52432 (30 μM, $n = 5$) on acetylcholine release in the hippocampus. Drugs were administered locally in the medial septum by retrograde dialysis. All values are shown as percent of baseline release (5 pretreatment samples). ∗significantly different from last baseline value in experiments with baclofen (Dunnett's multiple comparison, $P < 0.05$). Kainic acid (5 μM) was administered in the medial septum following CGP 52342 administration in order to validate the function of the dialysis probe in the medial septum. +: significantly different from the last value preceding kainic acid administration (Dunnett's multiple comparison, $P < 0.05$).

In order to verify the functioning of the dialysis probes, we administered kainic acid (5 μM, Fig. 2) in the medial septum. Intraseptal administration of kainic acid increased acetylcholine release in the hippocampus in previous studies performed with the present experimental design (Moor et al., 1996). Kainic acid induced a rapid increase in hippocampal acetylcholine release to 185 ± 8% of baseline after 15 min of administration ( $\chi^2(4) = 12.7$, $P < 0.05$).

### 3.2. Ligands of benzodiazepine binding site

Midazolam was chosen as an antagonist at the benzodiazepine binding because it is known to decrease acetylcholine release in the hippocampus effectively, and because it is soluble in aCSF, it can be administered via the dialysis probe. Midazolam (100 μM) perfusion in the medial septum did not affect acetylcholine release in the hippocampus significantly ( $\chi^2(10) = 17.4$, n.s.), although a small tendency towards a decrease was present ($n = 7$, Fig. 3). In contrast, perfusion with the antagonist of the benzodiazepine binding site, flumazenil (100 μM, $n = 9$, Fig. 3), increased acetylcholine release in the hippocampus ( $\chi^2(10) = 31.5$, $P < 0.001$) to a maximum of 131 ± 8% after 75 min of administration.

### 3.3. GABA receptor agonists and antagonists in the hippocampus

Intrahippocampal administration of muscimol (100 μM, $n = 7$) failed to affect local acetylcholine release significantly ( $\chi^2(10) = 15.1$, n.s.), although a tendency towards an increase may have been present (not shown). Similarly, bicuculline administration (5 μM, $n = 5$) did not affect acetylcholine release in the hippocampus ( $\chi^2(10) = 6.3$, n.s.).

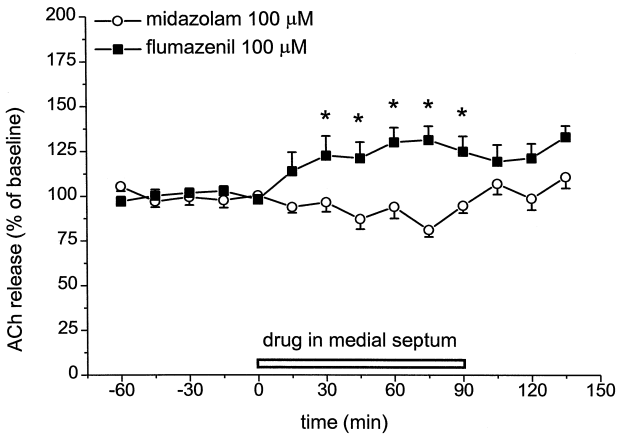


Fig. 3. Effects of the benzodiazepine midazolam (100 μM, $n = 7$) and the benzodiazepine antagonist flumazenil (100 μM, $n = 9$) in the medial septum on acetylcholine release in the hippocampus. Drugs were administered locally by retrograde dialysis. All values are shown as percent of baseline release (5 pretreatment samples). ∗significantly different from last baseline value in experiments with flumazenil (Dunnett's multiple comparison, $P < 0.05$).

Local administration of baclofen in the hippocampus (50 μM, $n = 5$, Fig. 4) did not affect acetylcholine release ( $\chi^2(10) =$ , n.s.). Acetylcholine release in this experiments was somewhat more irregular than in other experiments, and although there was an initial tendency towards a decrease (15, 30 and 45 min of administration, respectively), this tendency was not continued. The $GABA_B$ receptor antagonist CGP 52432 (30 μM, $n = 6$, Fig. 4)

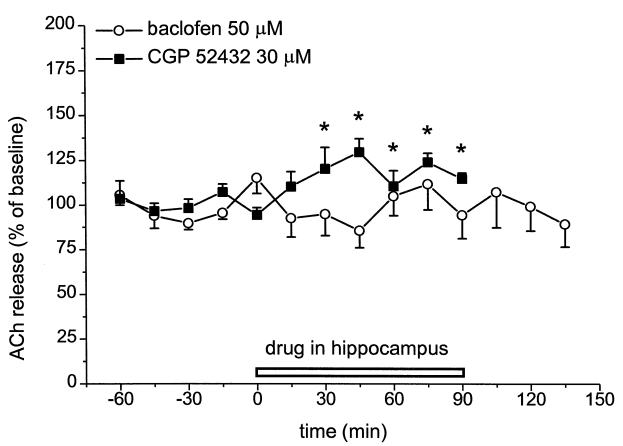


Fig. 4. Effects of intrahippocampal administration of the $GABA_B$ receptor agonist (±) baclofen (50 μM, $n = 5$) and the antagonist CGP 52432 (30 μM, $n = 6$) on acetylcholine release. The drugs were dissolved in aCSF and administered in the dialysis probe. All values are shown as percent of baseline release (5 pretreatment samples). ∗significantly different from last baseline value in experiments with CGP 52432 (Dunnett's multiple comparison, $P < 0.05$).

caused a small but significant increase in acetylcholine release ( $\chi^2(10) = 22.8,\ P < 0.05$).

## 4. Discussion

The present results show that GABA and GABA receptor agonists inhibit acetylcholine release in the hippocampus via $GABA_A$ receptors in the medial septum and vertical limb of the diagonal band of Broca. In this respect, the current results confirm previous findings obtained with a variety of methods. Our findings, however, provide new information about the site and dynamics of the interaction of GABA with hippocampal acetylcholine release.

### *4.1. $GABA_A$ receptor in the medial septum*

Muscimol administration in unaenesthetised animals induces vigorous behavioural activity (Moor et al., 1998). Several papers report an increase in locomotor activity after intraseptal muscimol (Allen and Crawford, 1984; Osborne, 1994), but decreased or unchanged locomotor activity following intraseptal muscimol has been reported in other studies (Chrobak et al., 1989; Durkin, 1992). Behavioural activity and arousal coincide with increases in hippocampal and cortical acetylcholine release (Dutar et al., 1979; Day et al., 1991; Acquas et al., 1996). Therefore, muscimol-induced behaviour could interfere with the direct inhibitory effect of muscimol. To evade this complication, experiments with muscimol were further conducted in halothane-anaesthetised rats. In anaesthetised animals, intraseptal muscimol reduced acetylcholine release in the hippocampus. Thus, acetylcholine release in the ventral hippocampus can be inhibited via septal $GABA_A$ receptors. The effect of intraseptal muscimol may have been underestimated in the present experiments because acetylcholine release was already decreased by the anaesthesia.

Administration of the $GABA_A$ receptor antagonist bicuculline caused a marked increase in acetylcholine release in the hippocampus. This result indicates that GABA exerts a tonic inhibitory effect on cholinergic septo-hippocampal neurons. This property of GABAergic inhibition in the medial septum was not discovered in earlier studies because intraseptal bicuculline failed to affect, as reflected by choline uptake or acetylcholine turnover in the hippocampus (Costa et al., 1983; Allen and Crawford, 1984; Wood, 1986; Walsh et al., 1993). The difference between the former and the present results is difficult to explain because the relation between acetylcholine output, as measured with microdialysis, and acetylcholine turnover and choline uptake is unclear. However, results obtained with other drugs indicate that some changes in cholinergic activity, particularly brief increases in acetylcholine release, may fail to alter-high affinity choline uptake and acetylcholine turnover rate. For example, intraseptal administration of the glutamate receptor agonist kainic acid did not affect acetylcholine turnover or high-affinity choline uptake, but increased acetylcholine release as measured by microdialysis (Brunello and Cheney, 1981; Wood, 1986; Moor et al., 1994, 1996).

### *4.2. $GABA_B$ receptors in the medial septum*

Administration of the $GABA_B$ receptor agonist baclofen into the medial septum induced a small decrease in hippocampal acetylcholine release. This result indicates that $GABA_B$ receptors in the medial septum mediate a minor component of the GABAergic inhibition of cholinergic neurons in the medial septum. In parallel, acetylcholine release in the striatum is also inhibited by GABA, mainly via $GABA_A$ receptors, but also via $GABA_B$ receptors (Anderson et al., 1993).

Only a small number of papers provide evidence for the function of $GABA_B$ receptors in the medial septum. Intraseptal baclofen induces a dose-related impairment in working memory (Stackman and Walsh, 1994) and inhibits bursting activity in the majority of septo-hippocampal neurons identified by antidromical stimulation of the fornix (Bassant et al., 1988). Intraseptal baclofen also reduces hippocampal high-affinity choline uptake to the same extent as muscimol (Walsh et al., 1993). In contrast, the basal acetylcholine turnover rate in the hippocampus was unaffected by intraseptal baclofen (Blaker et al., 1986).

Administration of the potent and selective $GABA_B$ receptor antagonist CGP 52432 (Lanza et al., 1993) in the medial septum did not affect acetylcholine release in the hippocampus. Thus, septal $GABA_B$ receptors on septo-hippocampal cholinergic neurons are not activated under the present experimental condition, in contrast to $GABA_A$ receptors.

Although the findings support a role for $GABA_B$ in the inhibition of septo-hippocampal neurons, the scope of $GABA_B$ receptor-mediated effects on acetylcholine appears to be limited. Possibly, the effects of $GABA_B$ receptor ligands were reduced by their effects on GABA release. $GABA_B$ receptors are known to function as inhibitory autoreceptors (Waldmeier et al., 1988). Therefore, baclofen is expected to reduce GABA release in the medial septum and consequently reduce GABAergic inhibition via $GABA_A$ receptors. This mechanism might interfere with the direct effect of baclofen on postsynaptic $GABA_B$ receptors.

### *4.3. Benzodiazepine binding sites in the medial septum*

Midazolam and diazepam are known to decrease acetylcholine release in the dorsal hippocampus after systemic and intraseptal administration (Dazzi et al., 1996; Imperato et al., 1993, 1994). In the present experiments, however, intraseptal administration of midazolam failed to affect acetylcholine release in the hippocampus significantly. Pilot experiments with injection of diazepam and midazolam

into the medial septum (instead of administration via the dialysis probe) yielded similar results. The discrepancy between the current and previous findings may reflect differential regulation of acetylcholine release in the ventral versus dorsal hippocampus, but factors such as differences in placement of the probe in the medial septum or in the methods of drug administration cannot be excluded.

Administration of the benzodiazepine antagonist flumazenil in the medial septum increased acetylcholine release in the hippocampus. Imperato et al. (1993, 1994) found that flumazenil increased acetylcholine release in the dorsal hippocampus in a dose-dependent manner after both systemic and intraseptal administration, and blocked the effect of diazepam on acetylcholine release. Also, high-affinity choline uptake in the hippocampus is reported to increase after intraseptal administration of flumazenil (Walsh et al., 1993). The effect of flumazenil was, however, much smaller in the current study than in these former studies.

The stimulation of acetylcholine release by flumazenil suggests that benzodiazepine binding sites are occupied by an endogenous ligand. However, flumazenil is not a neutral antagonist at the benzodiazepine binding site, and its action depends on the dose and the experimental model (see File and Pellow, 1986 for review). Accordingly, it has been suggested that flumazenil increases acetylcholine release because it acts as an inverse agonist at higher doses (Walsh et al., 1993; Imperato et al., 1994).

Taken together, the present results support the notion that benzodiazepine binding sites in the medial septum modulate acetylcholine release in the hippocampus. However, because midazolam failed to affect acetylcholine release in the hippocampus, and the effect of flumazenil was relatively small, the results do not support the idea that systemically administered benzodiazepines affect acetylcholine release in the hippocampus largely by acting on receptors in the medial septum. In this context, it should be noted that benzodiazepines are likely to affect acetylcholine release indirectly. Acetylcholine release in the hippocampus and cortex parallels the level of arousal and behavioural activity (Dutar et al., 1979; Day et al., 1991; Marrosu et al., 1995; Acquas et al., 1996). Further, benzodiazepines decrease spontaneous behaviour and arousal, and at least some benzodiazepine antagonists and inverse agonists are anxiogenic and stimulate spontaneous behaviour (File et al., 1982; Jackson and Nutt, 1992; Cole et al., 1995). Thus, ligands of the benzodiazepine binding site are likely to affect acetylcholine release through their effects on the level of arousal and behavioural activity.

### *4.4. GABA receptors in the hippocampus*

Intrahippocampal administration of muscimol and bicuculline, in the same concentrations that affected acetylcholine release upon intraseptal administration, did not affect acetylcholine release. Thus, $GABA_A$ receptors are not involved in the presynaptic modulation of acetylcholine release in the hippocampus.

Local administration of baclofen also failed to affect acetylcholine release in the hippocampus. Surprisingly, the $GABA_B$ receptor antagonist CGP 52432 caused a small but significant increase in acetylcholine release. This result indicates that GABAergic neurons in the hippocampus inhibit acetylcholine release via presynaptic $GABA_B$ receptors on cholinergic nerve terminals. To the best of our knowledge, this has not been demonstrated previously. However, $GABA_B$ receptors are known to act as presynaptic heteroreceptors at other synapses in the brain (e.g., Waldmeier et al., 1994) and to mediate presynaptic inhibition of acetylcholine release from parasympathetic neurons (Kataoka et al., 1994).

$GABA_B$ receptor-mediated inhibition appeared to be saturated in our experiments, because the agonist, but not the antagonist, affected acetylcholine release. This may seem improbable because further inhibition can not occur. However, local extracellular levels of acetylcholine were elevated by the neostigmine present in the aCSF. Acetylcholine in known to increase the firing-rate of hippocampal basket cells (Pitler and Alger, 1992), which are a probable source of GABA-mediated inhibition of acetylcholine release in the hippocampus. Thus, inhibition via $GABA_B$ receptors is likely to be increased under the present experimental conditions.

## 5. Conclusion

The present results indicate that septo-hippocampal cholinergic neurons are under continuous inhibition by endogenous GABA. GABA acts on hippocampal acetylcholine release largely via $GABA_A$ receptors in the medial septum, but also via $GABA_B$ receptors in the medial septum and on cholinergic nerve terminals in the hippocampus. GABAergic inhibition in the medial septum can be modulated by ligands of benzodiazepine binding sites, but the scope of the modulation is limited.

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