British Journal of Pharmacology (1997) 121, 1180–1186

Differential effects of the neuropeptide galanin on striatal acetylcholine release in anaesthetized and awake rats

[1]K. Antoniou, J. Kehr, K. Snitt & [2]S.O. Ögren

Division of Cellular and Molecular Neurochemistry, Department of Neuroscience, Karolinska Institute, 171 77 Stockholm, Sweden

1 In the present study the mechanisms were examined by which the neuropeptide galanin modulates the extracellular concentrations of striatal acetylcholine (ACh) in enflurane anaesthetized and in freely moving male rats by use of *in vivo* microdialysis and high performance liquid chromatography.

2 The perfusion of galanin through the microdialysis probe (0.3 nmol μl^{-1}, flow rate: 2 μl min^{-1}) caused a statistically significant increase in the basal striatal ACh levels in anaesthetized but a decrease in awake animals. No significant effect was revealed after a low dose (0.1 nmol μl^{-1}, flow rate: 2 μl min^{-1}) of galanin perfusion. Both the stimulating and inhibitory effects of galanin on basal ACh release were reversible.

3 The muscarinic antagonist scopolamine (0.1 mg kg^{-1}, subcutaneously (s.c.)) caused a significant increase in ACh release in both anaesthetized and awake animals.

4 The combination of galanin plus scopolamine attenuated the stimulant effect on ACh release caused by scopolamine alone in awake animals.

5 The putative galanin receptor antagonist M35 at 0.3 nmol μl^{-1} but not at 0.1 nmol μl^{-1} caused a significant reduction (20%) in ACh release, supporting the view that M35 at higher concentrations behaves as a partial agonist at the galanin receptor. When M35 (0.1 nmol μl^{-1}) was co-infused with galanin (0.3 nmol μl^{-1}) the galanin-evoked decrease in ACh release was completely blocked.

6 Taken together, these results indicate that galanin affects basal ACh release via stimulation of galanin receptors within the striatum. The mechanism involved is dependent on the anaesthesia procedure which may act via enhancement of γ-aminobutyric $acid_A$ ($GABA_A$) mediated transmission within striatal and/or output neurones. In addition, anaesthesia may also decrease the activity of glutamatergic striatal afferents. The results with M35 indicate that the role of galanin perfused in striatum is permissive in the normal rat. Furthermore, galanin is a potent inhibitory modulator of basal ACh release also in the striatum, as recently was shown in the ventral hippocampus in awake animals.

Keywords: Galanin; acetylcholine; striatum; scopolamine; M35; *in vivo* microdialysis

Introduction

Galanin, a 29 amino acid neuropeptide, has a widespread distribution in the CNS and in the periphery and it exerts many physiological effects (Tatemoto *et al*., 1983; Bartfai *et al*., 1993; Crawley, 1996). It has been shown to co-exist with several classical neurotransmitters in the brain (Melander *et al*., 1986). For instance, galanin immunohistochemistry has been shown to be present within a subpopulation of cholinergic neurones projecting from septum-basal forebrain complex to cortical and hippocampal areas in rats treated with high doses of colchicine (Melander *et al*., 1985; Senut *et al*., 1989). Galanin-acetylcholine co-existence is of particular interest since the forebrain cholinergic systems are involved in learning and memory processes in the rat (Hagan & Morris, 1988; Crawley & Wenk, 1989). In addition, the memory deficits associated with Alzheimer's disease have been suggested to involve a loss of cortical and hippocampal cholinergic innervation (Coyle *et al*., 1983). Thus, the presence of galanin within forebrain cholinergic neurones seems to be of potential importance in the pathology of Alzheimer's disease. An overexpression of galanin interneurones has been observed in the vicinity of the nucleus basalis in *post-mortem* brains from patients with Alzheimer's disease (Chan-Palay, 1988; Beal *et al*., 1990; Mufson *et al*., 1993; Gabriel *et al*., 1994). However, the mechanism behind the galanin hyperinnervation of the basal cholinergic neurones is still unknown.

Given that galanin seems to play a regulatory role on the cholinergic function, several studies have focused on the effect of galanin on the acetylcholine (ACh) release in different rat brain regions by use of both *in vitro* or *in vivo* techniques (Fisone *et al*., 1987; Bartfai *et al*., 1993; Pramanik & Ögren, 1993; Ögren *et al*., 1996) and tried to relate changes in cholinergic transmission with the behavioural effects of galanin in tasks related to learning and memory in the rat. Although galanin was shown to reduce the potassium-evoked release of [^{3}H]-ACh from ventral but not dorsal hippocampal slices from the rat and monkey (Fisone *et al*., 1987; 1991), our knowledge of the effects of galanin on the regulation of *in vivo* ACh release is still limited. By use of the microdialysis procedure, galanin administered intraventricularly (i.c.v.) to freely moving female rats, failed to reduce the basal ACh release in the ventral hippocampus (Fisone *et al*., 1987). However, galanin given i.c.v. (3 nmol) completely blocked the increase in ACh release in the ventral hippocampus evoked by systemic administration of the muscarinic antagonist scopolamine in the rat (Fisone *et al*., 1987). In a recent *in vivo* brain dialysis study, on the other hand, it was shown that galanin administered i.c.v. (0.32 nmol per rat) to the male rat significantly decreased the basal ACh release in the ventral hippocampus (Hiramatsu *et al*., 1996). In another study, in freely moving male rats, galanin given i.c.v. (3 nmol per rat) did not affect the basal ACh release in the ventral hippocampus, while perfusion of galanin (0.3 nmol μl^{-1}, flow rate: 1.25 μl min^{-1}) through the microdialysis probe in the ventral hippocampus produced a dose-dependent, reversible reduction in the ACh release (Ögren *et al*., 1996). In addition, galanin given i.c.v.

[1]Present address: Department of Experimental Pharmacology, Medical School, University of Athens, 11427 Athens, Greece.
[2]Author for correspondence.

or through the probe partially blocked the increase in ACh release induced by perfusion and s.c. administration of scopolamine (0.1 mg kg^{-1}). In the rat striatum, on the other hand, galanin has been shown to stimulate the basal release of ACh (Ögren & Pramanik 1991a,b; Amoroso *et al*., 1992; Pramanik & Ögren, 1992; 1993). In this structure, galanin given i.c.v., (3.12 nmol in 15 μl) was found to induce a transient increase (50%) in the basal ACh release in freely moving rats while a long lasting increase (60%) was seen only when the corticostriatal input was removed (Amoroso *et al*., 1992). The same authors found that scopolamine given s.c. produced an increase in the basal ACh release in cortically lesioned rats and that the co-administration of galanin (i.c.v.) with scopolamine (s.c.) resulted into an additive effect. Microdialysis experiments performed in the anaesthetized rats revealed that galanin administered i.c.v. (3 nmol in 10 μl) caused a statistically significant but short-lasting increase (100%) in the basal ACh in the striatum and a long lasting increase (100%) when galanin was perfused continuously through the microdialysis probe (Ögren & Pramanik, 1991a,b; Pramanik & Ögren, 1992). In contrast to the results obtained in awake animals (Amoroso *et al*., 1992), the results in the anaesthetized rats did not indicate any additive effect after the co-administration of galanin with scopolamine compared to that of scopolamine alone (Ögren & Pramanik, 1991b; Pramanik & Ögren, 1993). The above results indicated that galanin induces a prominent increase in the basal ACh release in the striatum in both anaesthetized and freely moving rats, but the results obtained after co-administration of galanin with scopolamine appeared to differ.

The purpose of the present study was to investigate further the effect of galanin on the basal release of ACh in the striatum in freely moving animals by use of a continuous perfusion of galanin through the microdialysis probe. In addition, the effect of galanin on the scopolamine-evoked ACh release was further analysed in order to examine whether anaesthesia is a (confounding) factor in the results observed previously. Furthermore, the effect of M35 (galanin(1-13)-bradykinin(2-9) amide), a putative galanin antagonist (Bartfai *et al*., 1992; Ögren *et al*., 1993; Kask *et al*., 1995), on the galanin-induced effect on ACh release was investigated, in order to define the mechanism of action of galanin in the striatum of awake rats. M35 has been shown to bind competitively to galanin receptors within the striatum (Bartfai *et al*., 1993) and to displace [^{125}I]-galanin from the rat striatal membranes in a concentration-dependent manner (Ögren *et al*., 1993).

Methods

Animals

Adult male Sprague-Dawley rats (age 10–12 weeks, weighing 280–310 g at the time of testing) were obtained from B&K Universal (Sweden). At least a five-day adaptation period to the animal maintenance facilities of the department was allowed before any treatment. The rats were housed, six by six, in standard plastic type IV Macrolon cages (57 × 35 × 19 cm, with 21 wood-cuttings as bedding) up to the time of surgery. They were maintained at an ambient room temperature of 19 ± 0.5°C with 40–50% relative humidity. A 12 h light/dark schedule (lights on at 06 h 00 min) was used throughout the experiment and the animals had free access to lab chow (Ewos R36, Sweden) and tap water up to time of each experiment.

Microdialysis

Microdialysis was carried out as described previously (Pramanik & Ögren, 1992; 1993) in freely moving and anaesthetized male rats. A microdialysis probe (CMA/12) of concentric design (outer diameter 0.5 mm, dialysing membrane 3 mm at the tip, mol. wt. cut-off: 20,000, CMA/Microdialysis, Sweden) was implanted into the right striatum under enflurane anaesthesia according to the following coordinates: AP + 1.3 mm, L + 2.2 mm, V − 6.2 mm (from the brain surface) (Paxinos & Watson, 1986). The probe was fixed to the skull by cold acrylic dental cement (Sevriton, DAB Dental, Sweden). After the operation the rats were removed from the anaesthesia and allowed to recover for 24 h in their home cages. After the recovery period (24 h), perfusion was initiated at a constant rate of 2 μl min^{-1} (for a period of 60 min) with Ringer solution (147 mM NaCl, 2.3 mM $CaCl_2$, 4 mM KCl, pH 6.1), containing 10 μM physostigmine sulphate. During this adaptation period no samples were collected.

In the anaesthetized rats the probe was fixed by a sterotaxic holder. After the operation, the perfusion was performed in the same way as in the freely moving animals. During the adaptation time (10 min) no samples were collected. In the experimental phase of both procedures, samples were collected by a CMA/170 Refrigerated Fraction Collector (CMA/Microdialysis). The dialysate was discarded during the first 20 min and then collected at 10 min intervals in small glass vials of 250 μl for a 60 min period before administration of galanin or scopolamine. Ten (or more when necessary) additional 10 min fractions were then sampled. Following the microdialysis session the position of the probe was regularly checked by inspection of sections from the frozen brains.

The average ACh content in the three 10 min consecutive fractions collected before administration of galanin and/or scopolamine (not corrected for recovery) was used as the basal level and expressed as 100%. The *in vitro* recovery of the microdialysis probe for ACh was in the range of 18–20% ($n = 10$). Galanin (porcine) was applied locally into the striatum, in freely moving rats, by perfusion through the microdialysis probe. Scopolamine was injected subcutaneously (s.c. in the scruff of the neck) either in freely moving rats or in anaesthetized rats.

H.p.l.c. (high performance liquid chromatography) assay

The acetylcholine content of the 10 min perfusates was measured by h.p.l.c. with a postcolumn immobilized-enzyme reactor and electrochemical detection as described previously (Fujimori & Yamamoto, 1987; Carter & Kehr, 1997). Briefly, acetylcholine and choline were first separated on a 100 × 2 mm polymeric reversed-phase column (BAS Co. Ltd., Tokyo, Japan) followed by conversion to betaine and hydrogen peroxide in a 2 × 5 mm enzyme reactor with immobilized acetylcholinesterase and choline-oxidase (BAS, Japan). Hydrogen peroxide was detected by a LC4B amperometric detector (Bioanalytical Systems Inc., West Lafayette, IN, U.S.A.) equipped with a platinum electrode operating at + 500 mV vs Ag/AgCl reference electrode. The h.p.l.c. system included a PM60 Pump (BAS, U.S.A.), a CMA/200 Refrigerated Microsampler (CMA/Microdialysis), a BAS LC4B electrochemical detector and a SP 4400 integrator (Spectra Physics, U.S.A.). The mobile phase consisted of 0.15 M sodium phosphate buffer, 1 mM octanesulphonic acid, 0.3 mM disodium-EDTA and 0.05% v/v Kathon CG (Rohm and Haas Co., PA, U.S.A.) adjusted to pH 8.5. The flow rate was 0.2 ml min^{-1}. The limit of detection for ACh was 0.23 pmol per 20 μl injection. The average extracellular basal levels of ACh in 20 μl fractions (not corrected for recovery) and in the presence of 10 μM physostigmine were 5.74 ± 0.67 pmol (means ± s.e.mean, $n = 16$) in the anaesthetized animals and 8.39 ± 0.45 pmol ($n = 46$) in the awake animals (for further details see the respective legend of each figure).

Peptide and compounds

Physostigmine sulphate (dissolved in the perfusion solution) and scopolamine hydrobromide (dissolved in saline (0.9% NaCl)) were obtained from Sigma (MO, U.S.A.). Scopolamine solution was prepared before use and was injected at a

volume of 2 ml kg^{-1}, s.c., in the neck. Porcine galanin (Bachem, Switzerland) was dissolved in artificial CSF (mM: NaCl 123.4, $NaHCO_3$ 23.4, KCl 2.4, KH_2PO_4 0.5, $CaCl_2.2H_2O$ 1.1, $MgCl_2.6H_2O$ 0.8, Na_2SO_4 0.5 and glucose 5.8, pH 7.1), and stored frozen (−20°C) in aliquot vials until use. Concentrations of galanin were calculated on the basis of the purity of the peptide. The vials were thawed on ice and brought to room temperature before the initiation of perfusion. This stock solution of galanin was diluted in Ringer solution (containing 10 μM physostigmine) and applied locally into the striatum by perfusion through the microdialysis probe. The chimeric peptide M35 was synthesized as described earlier (Land *et al*., 1991) and it was kindly provided by Prof. T. Barfai and Dr U. Langel, Stockholm University. M35 solutions were prepared by use of the same procedure as with galanin solutions. All other chemicals used were of analytical grade.

Statistical analysis

The data from the *in vivo* microdialysis studies (h.p.l.c. assays) were examined by a repeated-measures two-way analysis of variance (ANOVA). Fisher's protected least significant difference test (Fisher's PLSD-test) was used to analyse the statistical significance between the groups at different time points. A level of $P<0.05$ was accepted as evidence for a statistically significant effect.

Results

Effects of galanin on in vivo *basal ACh release in the striatum*

Galanin (0.3 nmol μl^{-1} and 0.1 nmol μl^{-1}, flow rate: 2 $\mu l\ min^{-1}$) was infused (60 min) through the microdialysis probe directly into the striatum. An increase in ACh release (about 60% of control value, $P<0.0001$ Fisher's PLSD-test), was seen with 0.3 nmol μl^{-1} galanin in anaesthetized rats (Figure 1). The effect appeared within 10 min after the initiation of infusion ($P<0.01$) and it lasted the entire perfusion time. After the cessation of galanin perfusion through the probe, a return was seen in the basal levels while during the next 20 min a decrease in the basal ACh release was observed ($P<0.01$) (Figure 1). No significant effect was observed after 0.1 nmol μl^{-1} galanin in the anaesthetized rats (data not shown).

In contrast, when galanin (0.3 nmol μl^{-1}, flow rate: 2 $\mu l\ min^{-1}$) was infused through the microdialysis probe (60 min) directly into the striatum of awake rats a decrease (about 40% of control value, $P<0.001$ Fisher's PLSD-test) was seen in the basal ACh levels (Figure 1). The effect appeared within 20 min after the initiation of infusion ($P<0.01$) and lasted the entire perfusion time (Figure 1). No significant effect was observed after 0.1 nmol μl^{-1} galanin in the awake rats (data not shown).

Effects of M35 on basal and galanin-induced ACh release in the striatum

M35 (0.3 nmol μl^{-1} and 0.1 nmol μl^{-1}, flow rate: 2 $\mu l\ min^{-1}$) was infused (60 min) through the microdialysis probe (60 min) directly into the striatum. M35 0.3 nmol μl^{-1} caused a small reduction (about 20%, $P<0.05$ Fisher's PLSD-test) of the basal ACh release in the striatum of awake animals within the first 20–40 min of perfusion (Figure 2). M35 0.1 nmol μl^{-1} failed to affect basal ACh release (Figure 2).

When M35 (0.1 nmol μl^{-1}, flow rate: 2 $\mu l\ min^{-1}$) was co-infused with galanin (0.3 nmol μl^{-1}, flow-rate: 2 $\mu l\ min^{-1}$) through the probe (60 min) in awake rats, M35 blocked fully the galanin-induced decrease in ACh release in the striatum ($P<0.001$, Fisher's PLSD-test) (Figure 2). This blocking effect, which was evident within 20 min ($P<0.01$, Fisher's PLSD-test), lasted the entire perfusion time and returned to baseline levels after the cessation of the perfusion through the probe (Figure 2).

Effects of scopolamine on basal in vivo *acetylcholine release in the striatum*

The basal release of endogenous ACh was increased by the muscarinic antagonist scopolamine (0.1 mg kg^{-1}, s.c.) in awake ($P<0.001$, Fisher's PLSD-test) as well as in anaesthetized animals ($P<0.001$, Fisher's PLSD-test) (Figure 3). In awake rats the effect of scopolamine appeared within 10 min ($P<0.05$, Fisher's PLSD-test) and lasted throughout the entire observation period, while in anaesthetized rats the effect ap-

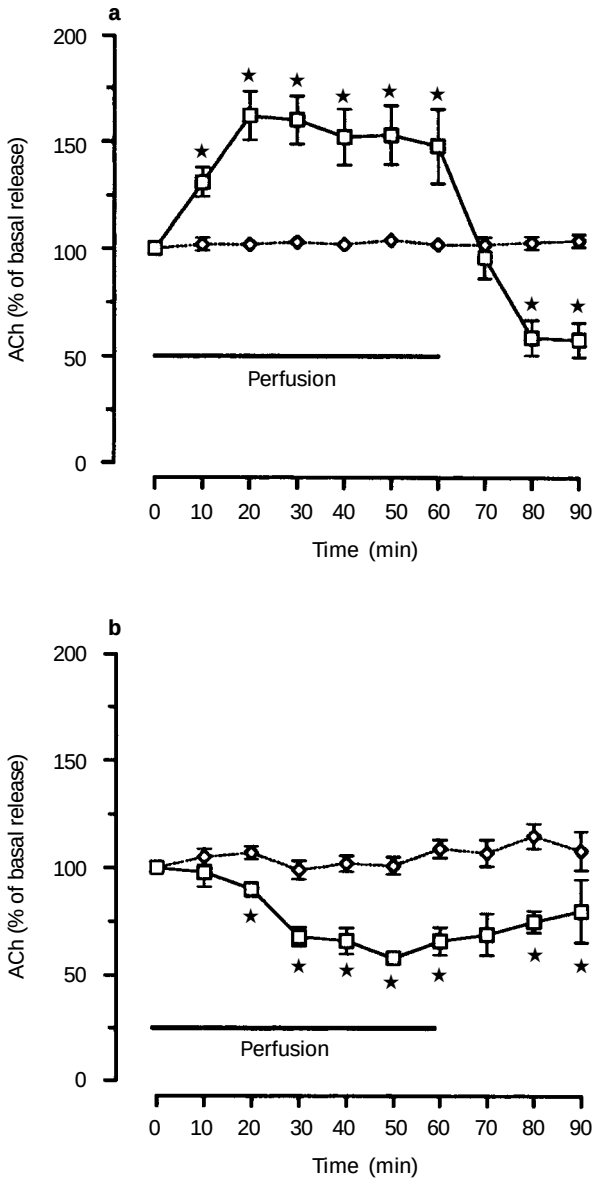

Figure 1 Effects of galanin on basal ACh release in striatum in (a) anaesthetized and (b) awake rats. (□) Galanin was perfused (0.3 nmol μl^{-1}, flow rate: 2 $\mu l\ min^{-1}$) for a period of 60 min into the striatum; (◇) effects of perfusion with Ringer solution. Each value represents the average ACh concentration in 10 min samples from two different groups of rats; vertical lines show s.e.mean. (a) Anaesthetized: Ringer, $n=4$, basal ACh level was 5.59 ± 0.53 pmol in 20 μl; galanin, $n=4$ basal ACh level was 5.46 ± 1.21 pmol in 20 μl. (b) Awake: Ringer, $n=8$, basal ACh level was 8.22 ± 1 pmol in 20 μl; galanin, $n=4$, basal ACh level was 5.29 ± 1.51 pmol in 20 μl. *$P<0.05$ vs all the corresponding time points for the respective control values (Fisher's PLSD-test).

peared within 20–40 min ($P<0.05$, Fisher's PLSD-test) and also lasted throughout the entire observation period. The increase of ACh release ranged from 70 to 90% of the baseline levels during the period of 40 to 100 min (Figure 3). It is apparent that there are no differences regarding the quantitative effects of scopolamine on extracellular ACh overflow in anaesthetized and awake animals (Figure 3).

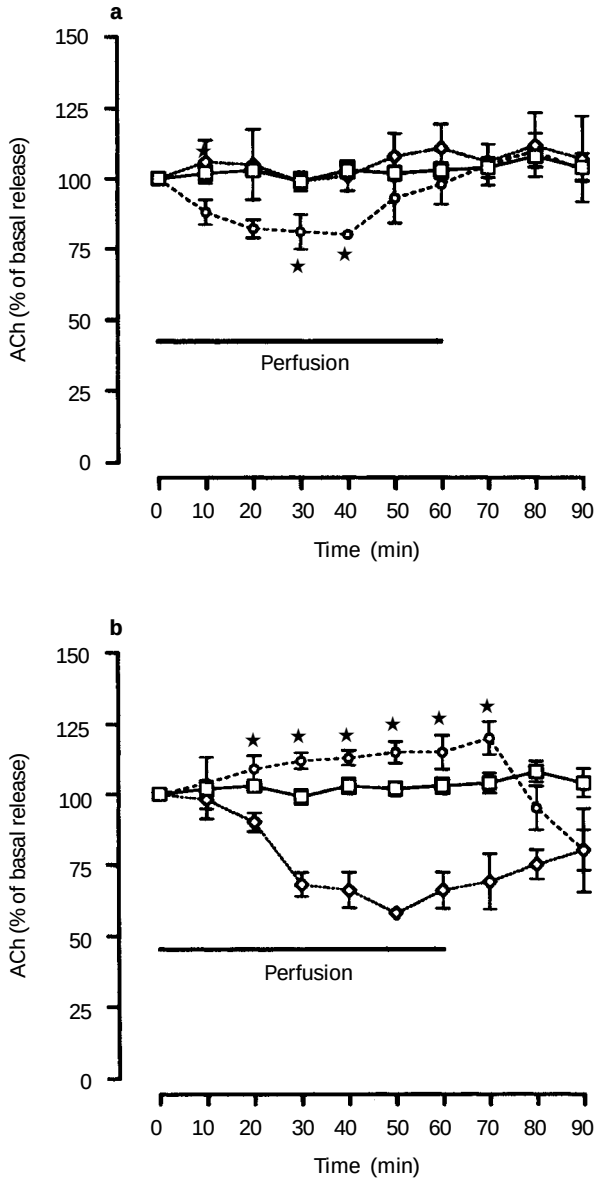

Figure 2 Effects of M35 and the combination of galanin plus M35 on the basal levels of striatal ACh in awake rats. (a) M35 was perfused (◇) (0.1 nmol μl^{-1} and (○) 0.3 nmol μl^{-1}, flow rate: 2 μl min^{-1}) for a period of 60 min into the striatum in awake rats; (□) infusion of Ringer solution. Each value represents the average ACh concentration in 10 min samples from three different groups of rats; vertical lines show s.e.mean. Ringer, $n=8$, basal ACh level was 8.22 ± 1 pmol in 20 μl. M35 (0.1 nmol μl^{-1}), $n=4$, basal ACh level was 9.44 ± 1.55 pmol in 20 μl. M35 (0.3 nmol μl^{-1}), $n=4$, basal ACh level was 6.73 ± 0.32 pmol in 20 μl. $^*P<0.05$ vs all the corresponding time points for M35 vs control values (Fisher's PLSD-test). (b) galanin (0.3 nmol μl^{-1}, flow rate: 2 μl min^{-1}) was coinfused with M35 (0.1 nmol μl^{-1}, flow rate: 2 μl min^{-1}) for a period of 60 min into the striatum. Each value represents the average ACh concentration in 10 min samples from three different groups of rats. (□) Ringer, $n=8$, basal ACh level was 8.22 ± 1 pmol in 20 μl. (◇) Galanin, $n=4$, basal ACh level was 5.29 ± 1.51 pmol in 20 μl. (○) M35 + galanin, $n=4$, basal ACh level was 7.51 ± 1.35 pmol in 20 μl. $^*P<0.05$ vs all the corresponding time points for galanin vs galanin + M35 values (Fisher's PLSD-test).

Effects of galanin on scopolamine-induced ACh release in the striatum

Previous experiments in the striatum, with anaesthetized rats, have shown that galanin given in combination with scopolamine did not modify the stimulating effect on the basal ACh release induced by systemic administration of this muscarinic antagonist (Pramanik & Ögren, 1993). In the present study a partial attenuation in the ACh release was seen after the co-administration of galanin (100 min, through the probe) and scopolamine (0.1 mg kg^{-1}, s.c., administration) when compared to the scopolamine alone ($P<0.05$ Fisher's PLSD-test) (Figure 4). Thus, galanin plus scopolamine induced a statistically significant decrease compared to

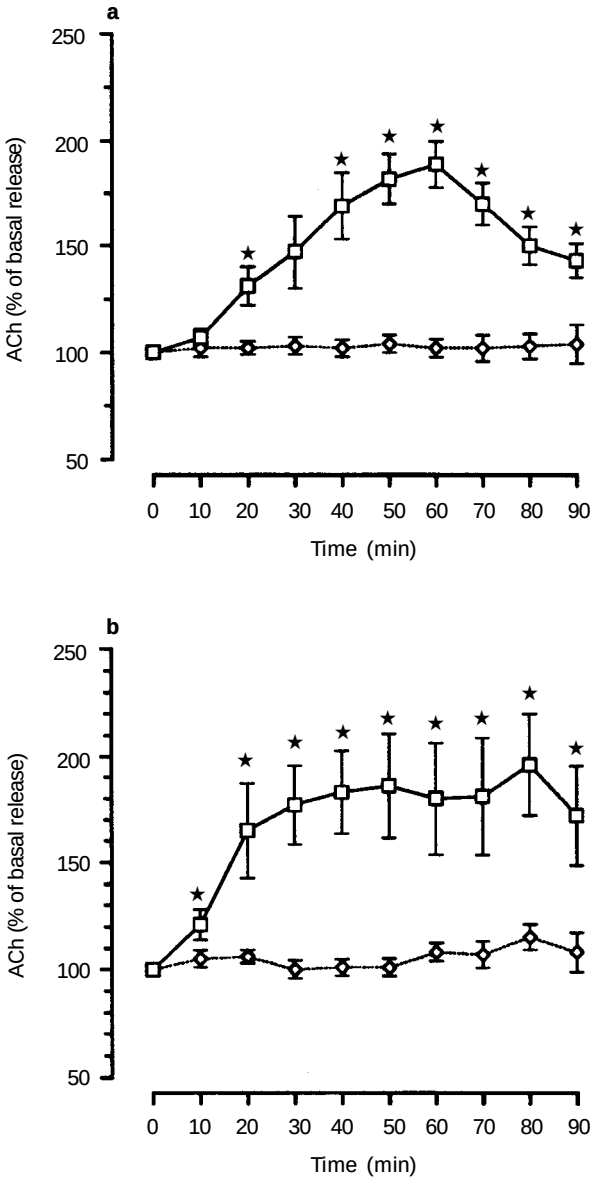

Figure 3 Effects of scopolamine on basal ACh release in striatum in both (a) anaesthetized and (b) awake rats. Scopolamine was administered s.c. in the neck (0.1 mg kg^{-1}). Each value represents the average ACh concentration in 10 min samples from two different groups of rats; vertical lines show s.e.mean. (a) Anaesthetized: (◇) Ringer, $n=4$, basal ACh level was 5.59 ± 0.53 pmol in 20 μl; (□) scopolamine, $n=6$ basal ACh level was 5.96 ± 1.25 pmol in 20 μl. (b) Awake: (◇) Ringer, $n=10$, basal ACh level was 9.8 ± 0.75 pmol in 20 μl; (□) scopolamine, $n=5$, basal ACh level was 8.77 ± 1.53 pmol in 20 μl. $^*P<0.05$ vs all the corresponding time points for the respective control values (Fisher's PLSD-test).

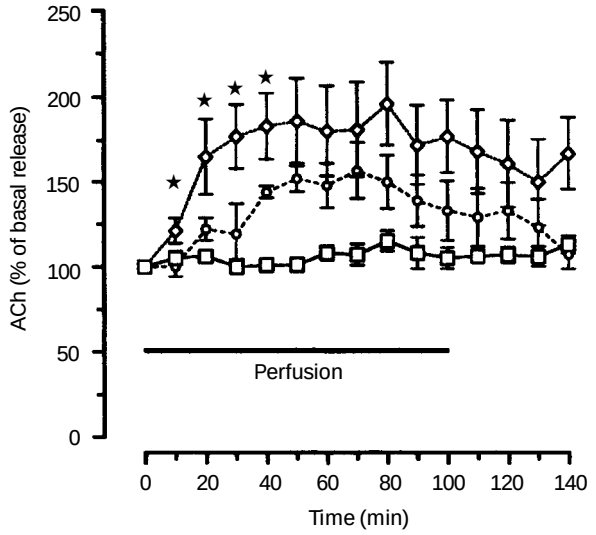

Figure 4 Effects of the combination of galanin plus scopolamine on the basal levels of striatal ACh in awake rats. Galanin was perfused (0.3 nmol μl^{-1}, flow rate: 2 $\mu l\ min^{-1}$) for a period of 100 min into the striatum and scopolamine 0.1 mg kg^{-1} was administered s.c. in the neck. Each value represents the average ACh concentration in 10 min samples from three different groups of rats; vertical lines show s.e.mean. (□) Ringer, $n=10$, basal ACh level was 9.8 ± 0.75 pmol in 20 μl; (◇) scopolamine, $n=6$, basal ACh level was 8.77 ± 1.53 pmol in 20 μl; (○) galanin + scopolamine, $n=5$, basal ACh level was 9.66 ± 0.19 pmol in 20 μl. $^*P<0.05$ vs all the corresponding time points for scopolamine vs galanin + scopolamine values (Fisher's PLSD-test).

the effect of scopolamine alone during the first 40 min ($P<0.05$, Fisher's PLSD-test) after the initiation of perfusion (Figure 4).

Discussion

The effects of galanin on both basal and scopolamine-evoked release or striatal ACh was examined in freely moving and anaesthetized rats by use of probes implanted into the medial part of striatum. The basal extracellular concentration of ACh in the striatum in freely moving rats was about 30% higher than that seen in the anaesthetized animals by use of the same procedure. A decrease in the ACh basal release by about 70% was found during chloral hydrate or halothane-induced anaesthesia (Bertorelli *et al*., 1990; Damsma & Fibiger, 1991). The above results indicate that enflurane anaesthesia similar to other anaesthetic agents reduces striatal cholinergic activity.

Galanin (0.3 nmol μl^{-1}, flow rate: 2 $\mu l\ min^{-1}$) delivered by perfusion to striatum, increased the extracellular levels of ACh by about 50–60% in anaesthetized rats while it decreased the basal ACh release by about 40% in awake animals. In anaesthetized rats, the stimulating effect lasted the entire perfusion time and it was reversed after the cessation of perfusion. Thus, the effect of galanin on ACh release was reversible after the cessation of perfusion followed by a rebound effect (a decrease in ACh release) observed only in anaesthetized rats. These results indicate that galanin is rapidly inactivated *in vivo* consistent with previous data (Ögren *et al*., 1996) and that the anaesthesia procedure plays a crucial role in the expression of galanin-induced effect on ACh release in striatum. The differential effects in awake and anaesthetized animals indicate that the anaesthetic agent enflurane may change the balance between excitatory and inhibitory neurotransmission within the striatum by reducing glutamatergic afferent excitatory input (Nicoll & Madison, 1982). Consistent with this notion is the observation that galanin given i.c.v. was shown to induce a long lasting increase in striatal ACh release in awake rats when the excitatory corticostriatal input was removed (Amoroso *et al*., 1992). However, the most important factor may be the enhancement of GABAergic inhibitory activity mediated via the $GABA_A$ type of receptors in the striatum (Harris *et al*., 1995). Potentiation of $GABA_A$ receptor-mediated transmission has been detected after both anaesthetic and subanaesthetic concentrations of enflurane (Mihic *et al*., 1994). Since the potentiation of GABA action by anaesthetics depends both on GABA concentrations, as well as, the different subunit compositions of $GABA_A$ receptor (Harris *et al*., 1995) the effects may be highly regional. The caudate putamen is known to express a large diversity of $GABA_A$ receptors (Wisden *et al*., 1992) which are expressed in the major classes of striatal interneurones and GABA-output neurones (Kawaguchi *et al*., 1995). In addition, anaesthesia most likely also enhances striatal dopaminergic input (Ståhle *et al*., 1990). It seems, therefore, likely that the action of galanin on ACh release depends on its relative effect on excitatory and inhibitory neurotransmitters in the striatum.

Taken together, the present results are consistent with previous experiments in anaesthetized rats showing an increase in ACh release both after i.c.v. administration or perfusion through the probe (Ögren & Pramanik, 1991a, b). On the other hand, galanin given i.c.v. was shown to induce a transient increase in striatal ACh release in awake female rats (Amoroso *et al*., 1992). These differential effects obtained in awake rats may depend on the different microdialysis probes used (concentric versus transversal probes), different solvents of galanin, different sexes of animals and in particular different routes of administration (perfusion at a constant rate versus i.c.v. injection).

The results with scopolamine confirm previous data showing a statistically significant increase in striatal ACh release after the systemic administration of scopolamine (Consolo *et al*., 1987; Ögren & Pramanik, 1991a,b; Murakami *et al*., 1995). The magnitude of the stimulant effect of scopolamine (0.1 mg kg^{-1}) was about the same in awake and anaesthetized animals (about 100% of the control level). This finding indicates that even if striatal cholinergic functions are suppressed during anaesthesia, the muscarinic-antagonist action on striatal ACh release is maintained. This insensitivity to anaesthesia probably reflects the fact that the muscarinic antagonist scopolamine acts directly via occupation of muscarinic autoreceptors located on both soma and terminal cholinergic interneurones. The stimulant effect of scopolamine on ACh release is most likely mediated by blockade of muscarinic autoreceptor sites mainly of the M_2 type expressed in striatal cholinergic interneurones (Bonner *et al*., 1987; Billard *et al*., 1995). A much more marked increase (800% of control) in the basal ACh release in the hippocampus was seen in freely moving rats after 0.1 mg kg^{-1} scopolamine (Ögren *et al*., 1996). Given that M_1 and M_4 autoreceptors are mainly expressed in hippocampal neurones (Vannuchi & Pepeu, 1995), the magnitude of the scopolamine-evoked release seems to be dependent on actions of multiple subtypes of muscarinic receptors. However, the differences between striatal cholinergic interneurones and the cholinergic terminal projections to the hippocampus probably play an important role in the mechanism of action of the muscarinic antagonist.

The perfusion of galanin through the probe with scopolamine (s.c.) attenuated the increase in the basal striatal ACh levels of scopolamine administered alone. In the deafferented-lesioned animals (where the glutamate uptake was reduced by 60% in the corticostriatal axis) an additive effect was seen after the co-administration of galanin i.c.v. with scopolamine (i.p.) Amoroso *et al*., 1992). Previous experiments in anaesthetized rats have shown that when scopolamine was given together with the galanin perfusion into the striatum no additive effect on ACh release was obtained (Ögren *et al*., 1992). Therefore, the present data indicate that the slight attenuation induced by galanin plus scopolamine in awake rats is a result of the inhibitory action of galanin on basal ACh release. Thus, the

mechanisms behind the effect of galanin on basal and scopolamine-induced ACh release appear to be the same. Furthermore, it seems clear that the mechanisms by which scopolamine and galanin affect ACh release differ (Pramanik & Ögren, 1993).

M35, a putative galanin receptor antagonist, at a dose of 0.1 nmol μl^{-1} did not induce any effect on striatal ACh release in awake animals but at a higher dose (0.3 nmol μl^{-1}) it induced a small reduction in the striatal ACh levels. This finding indicates that M35 at higher doses may behave as a (partial) agonist at striatal galanin receptors. When M35 0.1 nmol μl^{-1} was coinfused with galanin it was found to block fully the galanin-induced decrease in striatal ACh release in awake rats. A previous study in anaesthetized rats has shown an increase in striatal ACh release after the perfusion with M35 at 0.3 nmol μl^{-1} but not at 0.1 nmol μl^{-1} (Ögren *et al*., 1993). These results together with previous data indicate that the action of M35 on galanin receptors can be dose- or concentration-dependent and differ in various tissues. For instance, receptor binding studies in Rin m 5F cells, as well as, biochemical and electrophysiological studies have shown that M35 at higher doses behaves as a compound with partial agonistic properties at the galanin receptor (Kask *et al*., 1995). In contrast, M15 (galantide) failed to affect the basal striatal ACh release in anaesthetized animals and behaved as a full antagonist to galanin in the striatum (Ögren *et al*., 1993). The finding with M35 clearly demonstrates that the action of galanin on ACh release in awake rats is mediated via stimulation of striatal galanin receptors. These results also confirm earlier data in awake animals showing that galantide (M15, 9.26 nmol i.c.v.) could fully block the stimulation of ACh release by galanin in deafferented animals (Amoroso *et al*., 1992).

Galanin binding sites, as well as galanin containing neurones have been identified in the rat basal ganglia albeit in low numbers (Melander *et al*., 1988). However, recent studies in *post-mortem* human brain have shown a high density of [^{125}I]-galanin binding sites in the basal ganglia and in the substantia nigra zona compacta (Rodríguez-Puertas *et al*., 1997). There is also evidence for a large number of galanin positive cell bodies in the caudate nucleus and putamen in the monkey and human brain (Kordower *et al*., 1992). Therefore, it is feasible that changes in GAL receptor function may have profound effects on striatal cholinergic neurotransmission not only in the rat but also in the primate striatum.

Taken together, the present results indicate that galanin is a potent inhibitory modulator of basal ACh release in the striatum, as was recently shown in the ventral hippocampus in awake rats (Ögren *et al*., 1996). The mechanisms of action of galanin on the extracellular ACh levels in the striatum appear to be indirectly mediated via stimulation of galanin receptors located within the striatum.

This study was supported by the Swedish MRC (B96-14X-11588-01A), Åke Wiberg Stiftelse, Magnus Bergvall's Stiftelse and Loo och Hans Osterman's Stiftelse.

References

AMOROSO, D., GIROTTI, P., FISONE, G., BARTFAI, T. & CONSOLO, S. (1992). Mechanism of the galanin induced increase in acetylcholine release in vivo from striata of freely moving rats. *Brain Res*., **589,** 33 – 38.

BARTFAI, T., FISONE, G. & LANGEL, Ü. (1992). Galanin and galanin antagonists: molecular and biochemical perspectives. *Trends Pharmacol Sci*., **13,** 312 – 317.

BARTFAI, T., HÖKFELT, T. & LANGEL, Ü. (1993). Galanin – a neuroendocrine peptide. *Crit. Rev. Neurobiol*., **7,** 229 – 274.

BEAL, M.F., MACGARVEY, U.M. & SWARTZ, K.J. (1990). Galanin immunoreactivity is increased in the nucleus basalis of Meynert in Alzheimer's disease. *Ann. Neurol*., **28,** 157 – 161.

BERTORELLI, R., HALLSTRÖM, Å., HURD, Y., KARLSSON, A., CONSOLO, S. & UNGERSTEDT, U. (1990). Anaesthesia effects on in vivo acetylcholine transmission; comparisons of radioenzymatic and HPLC assays. *Eur. J. Pharmacol*., **175,** 79 – 83.

BILLARD, W., BINCH III, H., CROSBY, G. & MCQUADE, R.D. (1995). Identification of the primary muscarinic autoreceptor subtype in rat striatum as m2 through a correlation of in vivo microdialysis and in vitro receptor binding data. *J. Pharmacol. Exp. Ther*., **273,** 273 – 279.

BONNER, T.I., BUCKLEY, N.J., YOUNG, A. & BRANN, M. (1987). Identification of a family of muscarinic acetylcholine receptor genes. *Science*, **237,** 527 – 532.

CARTER, A.J. & KEHR, J. (1997). A microbore high-performance liquid chromatographic method for measuring acetylcholine in microdialysis samples: optimizing performance of platinum electrodes. *J. Chromatogr*., (in press).

CHAN-PALAY, V. (1988). Galanin hyper-innervates surviving neurons of the human basal nucleus of Meynert in dementias of Alzheimer's and Parkinson's disease: a hypothesis for the role of galanin in accentuating cholinergic-dysfunction in dementia. *J. Comp. Neurol*., **273,** 543 – 547.

CONSOLO, S., LADINSKY, H., VINCI, R., PALAZZI, E. & WANG, J.-X. (1987). An in vivo pharmacological study on muscarinic receptor subtypes regulating cholinergic neurotransmission in rat striatum. *Biochem. Pharmacol*., **36,** 3075 – 3077.

COYLE, J.T., PRICE, D.L. & DELONG, M.R. (1983). Alzheimer's disease: a disorder of cortical cholinergic innervation. *Science*, **219,** 133 – 141.

CRAWLEY, J.N. (1996). Galanin-acetylcholine interactions: relevance to memory and Alzheimer's disease. *Life Sci*., **58,** 2185 – 2199.

CRAWLEY, J.N. & WENK, G.L. (1989). Co-existence of galanin and acetylcholine: is galanin involved in memory processes and dementia? *Trends Neurosci*., **112,** 178 – 281.

DAMSMA, G. & FIBIGER, H.C. (1991). The effects of anaesthesia and hypothermia on interstitial concentrations of acetylcholine and choline in rat striatum. *Life Sci*., **48,** 2469 – 2474.

FISONE, G., BARTFAI, T., NILSSON, S. & HÖKFELT, T. (1991). Galanin inhibits the potassium-evoked release of acetylcholine and the muscarinic receptor-mediated stimulation of phosphoinositide turnover in slices of monkey hippocampus. *Brain Res*., **568,** 279 – 284.

FISONE, G., WU, C.F., CONSOLO, S., NORDSTRÖM Ö., BRYNNE, N., BARTFAI, T., MELANDER, T. & HÖKFELT, T. (1987). Galanin inhibits acetylcholine release in the ventral hippocampus of the rat: Histochemical, autoradiographic, in vivo, and in vitro studies. *Proc. Natl. Acad. Sci. U.S.A*., **84,** 7339 – 7343.

FUJIMORI, K. & YAMAMOTO, K. (1987). Determination of acetylcholine and choline in perchlorate extracts of brain tissue using liquid chromatography-electrochemistry with an immobilized-enzyme reactor. *J. Chromatogr*., **414,** 167 – 173.

GABRIEL, S.M., BIERER, L.M., DAVIDSON, M., PUROHIT, D.P., PERL, D.P. & HAROTUNIAN, V. (1994). Galanin-like immunoreactivity is increased in the post-mortem cerebral cortex from patients with Alzheimer's disease. *J. Neurochem*., **62,** 1516 – 1523.

HAGAN, J.J. & MORRIS, R.G.M. (1988). The cholinergic hypothesis of memory: a review of animal experiments. In *Handbook of Psychopharmacology*. ed. Iversen, L.L., Iversen, S.D. & Snyder, S.H., Vol. 20, pp. 237 – 323. London: Plenum Press.

HARRIS, R.A., MIHIC, S.J., DILDY-MAYFIELD, J.E. & MACHU, T.K. (1995). Actions of anesthetics on ligand-gated ion channels: role of receptor subunit composition. *FASEB J*., **9,** 1454 – 1462.

HIRAMATSU, M., MORI, H., MURASAWA, H. & KAMEYAMA, T. (1996). Improvement by dynorphin A(1-13) of galanin-induced impairment of memory accompanied by blockade of reductions in acetylcholine release in rats. *Br. J. Pharmacol*., **118,** 255 – 260.

KASK, K., BERTHOLD, M., BOURNE, J., ANDELL, S., LANGEL, Ü. & BARTFAI, T. (1995). Binding and agonist/antagonist actions of M35, galanin (1-13)-bradykinin (2-9) amide chimeric peptide, in Rin m 5F insulinoma cells. *Regul. Peptides*, **59,** 341 – 348.

KAWAGUCHI, Y., WILSON, C.J., AUGOOD, S.J. & EMSON, P.C. (1995). Striatal interneurones: chemical, physiological and morphological characterization. *Trends Neursci*., **18,** 527 – 535.

KORDOWER, J.H., LE, H.K. & MUFSON, E.J. (1992). Galanin immunoreactivity in the primate central nervous system. *J. Comp. Neurol.*, **319,** 479–500.

LAND, T., LANGEL, Ü., LÖW, M., BERTHOLD, M., UNDÉN, A. & BARTFAI, T. (1991). Linear and cyclic N-terminal galanin fragments and analogs as ligands at the hypothalamic galanin receptor. *Int. J. Pept. Protein Res.*, **38,** 267–272.

MELANDER, T., HÖKFELT, T., RÖKAEUS, Å., CUELLO, A.C., OERTEL, W.H., VERHOFSTAD, A. & GOLDSTEIN, M. (1986). Coexistence of galanin-like immunoreactivity with catecholamines, 5-hydroxytryptamine, GABA and neuropeptides in the rat CNS. *J. Neurosci.*, **6,** 3640–3654.

MELANDER, T., KÖHLER, C., NILSSON, S., HÖKFELT, T., BRODIN, E., THEODORSSON, E. & BARTFAI, T. (1988). Autoradiographic quantitation and anatomical mapping of ^{125}I-galanin binding sites in the rat central nervous system. *J. Chem. Neuroanat.*, **1,** 213–233.

MELANDER, T., STAINES, W.A., HÖKFELT, T., RÖKAEUS, A., ECKENSTEIN, F., SALVATERRA, P.M. & WAINER, B.H. (1985). Galanin-like immunoreactivity in cholinergic neurons of the septum-basal forebrain complex projecting to the hippocampus of the rat. *Brain Res.*, **360,** 130–138.

MIHIC, S.J., MCQUILKIN, S.J., EGER, E.I., IONESCU, P. & HARRIS, R.A. (1994). Potentiation of gamma-aminobutyric acid type A receptor-mediated chloride contents by novel halogenated compounds correlates with their abilities to induce general anesthesia. *Mol. Pharmacol.*, **46,** 851–857.

MUFSON, E.J., COCHRAN, E., BENZING, W. & KORDOWER, J.H. (1993). Galaninergic innervation of the cholinergic vertical limb of diagonal band (Ch2) and bed nucleus of the stria terminalis in aging, Alzheimer's disease and Down's syndrome. *Dementia*, **4,** 237–250.

MURAKAMI, Y., MATSUMOTO, K., OHTA, H. & WATANABE, H. (1995). Effects of oxotremorine and pilocarpine on striatal acetylcholine release as studied by brain dialysis in anesthetized rats. *Gen. Pharmacol.*, **27,** 833–836.

NICOLL, R.A. & MADISON, D.V. (1982). General anesthetics hyperpolarize neurons in the vertebrate central nervous system. *Science*, **217,** 1055–1057.

ÖGREN, S.O., KEHR, J. & SCHÖTT, P.A. (1996). Effects of ventral hippocampal galanin on spatial learning and on in vivo acetylcholine release in the rat. *Neuroscience*, **75,** 1127–1140.

ÖGREN, S.O., PRAMANIK, A., LAND, T. & LANGELL, Ü. (1993). Differential effects of the putative galanin receptor antagonists M15 and M35 on striatal acetylcholine release. *Eur. J. Pharmacol.*, **242,** 59–64.

ÖGREN, S.O. & PRAMANIK, A. (1991a). Galanin: regulation of cholinergic function and behaviour. In *Cholinergic Basis for Alzheimer Therapy*. ed. Becker, R. & Giacobini, E. pp. 193–199. Boston: Birkhäuser.

ÖGREN, S.O. & PRAMANIK, A. (1991b). Galanin stimulates acetylcholine release in the rat striatum. *Neurosci. Lett.*, **128,** 253–256.

PAXINOS, G. & WATSON, C. (1986). *The Rat Brain in Sterotaxic Coordinates*. Australia: Academic Press.

PRAMANIK, A. & ÖGREN, S.O. (1993). Galanin stimulates striatal acetylcholine release via a mechanism unrelated to cholinergic receptor stimulation. *Regul. Pept.*, **45,** 353–362.

PRAMANIK, A. & ÖGREN, S.O. (1992). Galanin-evoked acetylcholine release in the rat striatum is blocked by the putative galanin antagonist M15. *Brain Res.*, **574,** 317–319.

RODRÍGUEZ-PUERTAS, R., NILSSON, S., PASCUAL, J., PAZOS, A. & HÖKFELT, T. (1997). ^{125}I-galanin binding sites in Alzheimer's disease: increase in hippocampal subfields and a decrease in the caudate nucleus. *J. Neurochem.*, **68,** 1106–1113.

SENUT, M.C., MENETREY, D. & LAMOUR, Y. (1989). Cholinergic and peptidergic projection from the medial septum and the nucleus of the diagonal band of Broca to dorsal hippocampus, cingulate cortex and olfactory bulb: a combined wheatgerm agglutinin-apohorseradish peroxidase-gold immunohistochemical study. *Neuroscience*, **30,** 385–403.

STÅHLE, L., COLLIN, A.-K. & UNGERSTEDT, U. (1990). Effects of halothane anaesthesia on extracellular levels of dopamine, dihydroxyphenylacetic acid, homovanillic acid and 5-hydroxyindolacetic acid in rat striatum: A microdialysis study. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **342,** 136–140.

TATEMOTO, K., RÖKAEUS, Å., JÖRNVALL, H., MCDONALD, T.J. & MUTT, V. (1983). Galanin- a novel biologically active peptide from porcine intestine. *Fedn. Eur. Biochem. Socs. Lett.*, **164,** 124–128.

VANNUCCHI, M.G. & PEPEU, G. (1995). Muscarinic receptor of acetylcholine release from rat cerebral cortex and hippocampus. *Neurosci. Lett.*, **190,** 53–56.

WISDEN, W., LAURIE, D.J., MONYER, H. & SEEBURG, P.H. (1992). The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. *J. Neurosci.*, **12,** 1040–1062.

(Received January 2, 1997
Revised March 11, 1997
Accepted April 3, 1997)