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Impulse flow dependency of galanin release *in vivo* in the rat ventral hippocampus

(inhibitory neuropeptide/electrical stimulation/microdialysis/galanin release *in vitro*/acetylcholine)

SILVANA CONSOLO*, GIANNI BALDI*, GIOVANNI RUSSI*, GIANLUCA CIVENNI*, TAMAS BARTFAI†, AND ANNAMARIA VEZZANI*

*Istituto di Ricerche Farmacologiche "Mario Negri," 20157 Milan, Italy; and †Department of Neurochemistry and Neurotoxicology, Arrhenius Laboratories, Stockholm University, S-10691 Stockholm, Sweden

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ABSTRACT **Using microdialysis and a sensitive RIA, we have studied the *in vivo* release of the neuropeptide galanin (GAL) from the ventral hippocampus of freely moving rats. The spontaneous outflow of GAL-like immunoreactivity (GAL-LI) (1.8 ± 0.3 fmol per ml per 20 min) was dependent on the presence of extracellular Ca^{2+} and was inhibited by tetrodotoxin. Evoked release induced by infusion of KCl (60 mM) or veratridine (148 μM) was also Ca^{2+}-dependent and sensitive to tetrodotoxin. Electrical stimulation of the ventral limb of the diagonal band nuclei induced a frequency-dependent (50–200 Hz) and tetrodotoxin-sensitive overflow of GAL-LI in the hippocampus. *In vitro* GAL-LI release (1.0 ± 0.02 fmol per ml per 5 min), studied in slices of rat ventral hippocampus, was also Ca^{2+}-dependent and was increased in a concentration-dependent manner by KCl depolarization. This study demonstrates the release of the neuropeptide GAL in the rat central nervous system. The *in vivo* release is related to the activity of the cholinergic GAL-LI-containing cells in the septal diagonal band nuclei. The results are discussed in relation to the coexistence of GAL and acetylcholine within the septal/diagonal band complex.**

Galanin (GAL) is a neuropeptide with widespread distribution in the endocrine and peripheral and central nervous systems (1–3) where it coexists with several classical neurotransmitters and peptides (3, 4). It appears to be involved in a variety of physiological processes including hormone secretion, neuronal activity, and smooth muscle contractility (4, 5).

Recent studies have reported on the effects of GAL in memory-related behaviors. The peptide impairs acquisition in the Morris swim maze (6) and in the delayed alternation T test (7), while the chimeric peptide GAL receptor antagonist M35 [galanin-(1–13)–bradykinin (2–9)] improved performance in the Morris swim test (8). These effects are believed to be exerted through interactions with the cholinergic forebrain neurons originating in the septal diagonal band nuclei and projecting to the hippocampus. Immunohistochemical and *in situ* hybridization studies have shown that GAL is synthesized and stored in the septal hippocampal cholinergic neurons in rodents and monkeys (9, 10). Actually, GAL is the only peptide known to coexist with acetylcholine (AcCho) in the forebrain neurons. GAL receptor autoradiography in combination with septal lesions indicates that in the ventral hippocampus of the rat these receptors are postsynaptic or are localized on the cholinergic nerve terminals (9).

Exogenous GAL inhibits the evoked release of AcCho both *in vivo* and *in vitro* and several GAL receptor antagonists reverse this effect (11–13). It also exerts an inhibitory effect on the postsynaptic muscarinic stimulation of inositol phospholipid production in the ventral hippocampus (14). On the other hand, the contribution of endogenous GAL to these effects has still not been tested. The presence of GAL in neurons, as shown by immunocytochemistry, cannot be taken as proof that the peptide is indeed released in the extracellular space, so it remains to be demonstrated whether endogenous GAL is released in the ventral hippocampus in response to appropriate stimuli. This knowledge is of scientific and clinical relevance because it would define this neuropeptide as a putative neurotransmitter and its role in physiological processes could then be investigated directly. This could possibly lead to validation of the pharmacological effects of GAL receptor antagonists that are expected to be useful in the treatment of Alzheimer type senile dementia, depression, and some eating disorders (7, 11, 12, 15).

In the present study we used the microdialysis technique coupled with RIA to study the basal and evoked *in vivo* release of GAL in the ventral hippocampus. The depolarizing agents applied to the ventral hippocampus or electrical stimulation of the cell bodies in the diagonal band nuclei resulted in Ca^{2+}- and tetrodotoxin (TTX)-dependent release of endogenous GAL. We also evaluated the *in vitro* release of GAL from slices of ventral hippocampus and demonstrated its dependence on Ca^{2+}- and KCl-induced depolarization.

MATERIALS AND METHODS

Animals. Female Sprague–Dawley rats (200–250 g, CD-COBS; Charles River Breeding Laboratories) were used. The experimental protocols were approved by an intramural review committee and experiments were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (16, ‡).

***In Vivo* Release.** For the microdialysis experiments, rats under Equithesin anesthesia [1% pentobarbital/4% (vol/vol) chloral hydrate; 3.5 ml/kg, i.p.] were placed in a stereotaxic frame. A guide cannula was implanted vertically as described (17) into the ventral hippocampus of one side at the following coordinates: nose bar −2.5; 5.0 mm posterior to the bregma, 4.8 mm lateral to the midline, and 4 mm below the surface of the dura mater (18). In some experiments, the dialysis probes were implanted in the ventral hippocampus of both sides.

On the day after cannula implantation, each rat was placed in a Plexiglas cage and a vertical dialysis probe with a 3-mm exchanging membrane (CMA10, Carnegie Medicin AB, Stockholm) was positioned inside the cannula protruding 3 mm. The probe was perfused at the constant rate of 5 μl/min with Ringer's solution (A) (147 mM NaCl/2.2 mM $CaCl_2$/4 mM KCl) adjusted to pH 7.4 with 1 M NaOH and supple-

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Abbreviations: GAL, galanin; GAL-LI, galanin-like immunoreactivity; TTX, tetrodotoxin; AcCho, acetylcholine.
‡European Economic Community Council, Directive 86/609, OJ L 358, 1, December 12, 1987.

mented with 0.2% bovine serum albumin and 0.03% bacitracin, respectively, to reduce the unspecific binding of the peptide and the activity of endogenous peptidases (19). The perfusate was discarded during the first 40 min to allow the GAL output to reach a steady baseline and then collected at 20-min intervals in 1.5-ml test tubes (Eppendorf), immediately frozen, and stored at −80°C until the GAL-like-immunoreactivity (GAL-LI) content was assayed by RIA. In some experiments, the ionic composition of the Ringer's solution was varied; i.e., the KCl concentration was raised to 60 mM and the NaCl concentration was lowered proportionally.

At the end of the release experiments, the placement of the dialysis probes was verified histologically by staining for Nissl substance.

To estimate the *in vitro* recovery of GAL through the membrane, the dialysis fiber was perfused at 5 μl/min with Ringer's solution identical to that used for the *in vivo* experiments and placed in 5 ml of the same medium at 37°C containing GAL (1 pmol/ml). After a 40-min washout, three 20-min samples were collected. The mean recovery (i.e., the mean concentration in the perfusate as a percentage of the concentration of the solution outside the fiber) was 7.0 ± 0.5% (n = 3 different fiber units) and was independent of the concentration in the external medium. Individual probes showed stable and reproducible relative recovery.

Electrode Implantation. Bipolar electrodes were constructed of fine nichrome wire (each wire, 62.5 μm in diameter; Plastic One, Roanoke, VA) and their tips were 0.2 mm apart. Under Equithesin anesthesia, rats were stereotaxically implanted bilaterally with the electrodes in the ventral limb of the diagonal band at the following coordinates from bregma: nose bar −2.5; AP +0.8; L ± 0.6; H −8.3. The electrode leads were attached to a multipin socket and fixed to the skull with dental acrylic. All solder joints were tested for continuity by a check of impedance. The same day the electrodes were implanted, two guide cannulae were positioned bilaterally in the ventral hippocampus for subsequent dialysis probe insertion, as described above.

Diagonal Band Nuclei Stimulation. Three days after implantation of the electrodes, each rat was put in a Plexiglas cage (as for release experiments) and the skull socket was mated with a plug connected to an electrical stimulator. The dialysis probes were bilaterally positioned in the ventral hippocampus and perfused with Ringer's solution as described above. After a 40-min washout and four consecutive 20-min samples (baseline), septal tetani (10 0.5-ms 10-V pulses, at 50–200 Hz and 140 μA) were applied for 10 s and repeated at 2-min intervals for 20 min (20). A 20-min fraction was collected during electrical stimulation followed by two consecutive 20-min samples. The perfusate samples were immediately frozen on dry ice and stored at −80°C until assayed.

***In Vitro* Release.** The rats were decapitated, the brains were rapidly removed from the skull, and the hippocampi of both hemispheres were dissected out at 4°C. After cutting the hippocampus with a blade midway and perpendicular to its longitudinal axis, the temporal pole was removed and used for release experiments. The ventral hippocampi were sliced in a coronal plane at a thickness of 0.3 mm with a McIlwain tissue chopper. The slices were pooled and incubated for 30 min at 37°C in 5 ml of oxygenated Ringer's buffer (B) (140 mM NaCl/5 mM KCl/1.2 mM $CaCl_2$/1.2 mM $MgSO_4$/1.2 mM sodium phosphate/11 mM $NaHCO_3$/10 mM glucose/0.2% bovine serum albumin/0.03% bacitracin, pH 7.4). The slices were placed in a set of parallel basket-shaped sieves with perforated bottoms [five slices per sieve; ≈13 mg (wet tissue weight)]. The sieves were then transferred to subsequent wells containing 500 μl of oxygenated Ringer's solution at 37°C (48-well tissue culture clusters; Costar).

After discarding the initial 20-min wash-out to allow for a stable baseline, the medium was collected at 5-min intervals for 30 min. To assess the effect of neuronal depolarization on GAL-LI efflux, the slices were challenged for 10 min with depolarizing concentrations of KCl (25, 50, and 100 mM) and the NaCl concentration was proportionally reduced. To check for the Ca^{2+} dependence of the spontaneous and 50 mM KCl-induced release, Ca^{2+} was omitted and 1 mM EGTA was added to the Ringer's solution for 20 min after a 10-min baseline. The collected medium (100 μl) was immediately frozen in 1.5-ml test tubes and stored at −80°C until assayed.

RIA. GAL-LI was determined by RIA. Assays were performed at +4°C in the tubes used for collecting the dialysate or the *in vitro* release medium as described in detail (21). Rat GAL (22) was used for preparing standards (0.25–128 fmol/ml), which were diluted with a Ringer's solution identical to that used for release experiments. The antiserum (diluted 1:130,000 in 0.1 M barbital buffer containing 0.2% bovine serum albumin, pH 8.6) was raised against rat GAL in a rabbit. No significant molar cross-reactivity was observed with various hormones and neuropeptides, as described in detail (23).

The nonspecific binding was determined in the standards and samples, and was ≈8% of the total radioactivity. The antibody-bound radioactivity of each sample was determined and the assay results were calculated on a computer (B/B_0 vs. logarithm concentration). The assay was sensitive to GAL at <1 fmol/ml (see Fig. 1). We set the detection limit of the assay at 0.5 fmol/ml, which is the amount of peptide that displaces 10 ±2% of specific tracer binding to the antiserum.

Chemicals and Antisera. Veratridine, bacitracin, bovine serum albumin, TTX, and EGTA were purchased from Sigma. Rat ^{125}I-labeled galanin (specific activity ≈1100 Ci/mmol; 1 Ci = 37 GBq) was purchased from Peninsula Laboratories (Belmont, CA). Rat galanin was purified by Ulo Langel and Tamas Bartfai (Arrhenius Laboratory, Department of Neurochemistry and Neurotoxicology, University of Stockholm); rat galanin antiserum was kindly supplied by Steven M. Gabriel (the Mount Sinai Medical Center, New York). Sheep anti-rabbit antibody-coated Sepharose suspension was purchased from Pharmacia.

Statistical Analysis. The results were calculated as GAL-LI concentration (fmol/ml) in a 20-min dialysate fraction or in 5-min samples, respectively, for *in vivo* and *in vitro* experiments. The effect of treatments was analyzed by one-way analysis of variance followed by Dunnett's or Tukey's test for unconfounded means.

RESULTS

Before animal experiments were started, the sensitivity of the RIA was optimized. The dilution of the antiserum was adjusted to produce specific binding of the radioligand in the absence of unlabeled peptide (B_0) amounting to 30 ± 3% (n = 7) of the total radioactivity added. The amount of peptide needed to displace 50% of specific tracer binding to the antiserum (IC_{50}) was 12.0 ± 0.8 fmol/ml (n = 7) (see also Fig. 1). To further validate our RIA procedure for assessing endogenous GAL-LI, we pooled dialysate samples from the ventral hippocampus obtained from probe infusion with 37 μM veratridine. The pooled perfusates were then divided into 50-, 100-, 200-, and 400-μl fractions, lyophilized, resuspended in 100 μl of Ringer's solution, and assayed by RIA. Displacement of tracer binding was comparable to that obtained with synthetic GAL standards (Fig. 1). In addition, no GAL-LI was detected by RIA when the release was measured in cerebellum, a brain area previously shown to be devoid of GAL (data not shown).

***In Vivo* Release.** The spontaneous efflux of GAL-LI from the ventral hippocampus of freely moving rats was steady over the 180-min measurement period and amounted to 1.8 ± 0.3 fmol per ml per 20 min (n = 14) (data not shown). The basal efflux of GAL-LI was strongly reduced (<0.5 fmol/ml) when the dialysis probe was perfused with a Ringer's solution not

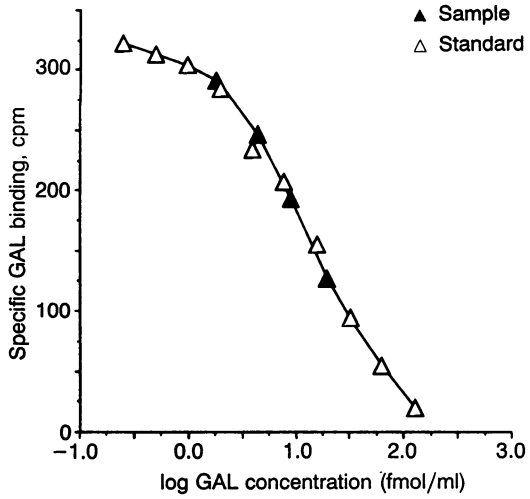

FIG. 1. GAL standard curve obtained using the RIA. The curve represents the mean of triplicate determinations (SEM was ≤3% of each mean value). The vertical axis denotes the net cpm (cpm minus blank) representing the specific binding of radioligand in the presence of added unlabeled GAL. The IC_{50} was 12.5 fmol/ml and the B_0 was 345 net cpm. The horizontal axis represents the logarithmic concentration of added synthetic GAL. Open and solid triangles represent standard and dialysate samples, respectively.

containing Ca^{2+} but supplemented with 1 mM EGTA (Fig. 2*A*) or containing 1 μM TTX, a blocker of voltage-dependent Na^+ channels (Fig. 2*B*).

Veratridine, a Na^+-channel opener, when infused in the ventral hippocampus through the dialysis probe for 40 min after an 80-min baseline, dose-dependently stimulated hippocampal GAL-LI outflow (Table 1). Peak increases of GAL-LI outflow were attained during the first 20 min of perfusion with 37, 74, and 148 μM veratridine and amounted to 236, 378, and 626%, respectively. The effects persisted for the next 20 min. The releasing effect of 148 μM veratridine was completely antagonized by 1 μM TTX added to the perfusion solution 20 min before and during a 40-min veratridine stimulation up to the end of the experiment (Fig. 3*A*).

When neuronal depolarization was induced by 40-min perfusion with 60 mM KCl, GAL-LI outflow increased 389% above the baseline in the first 20 min of potassium

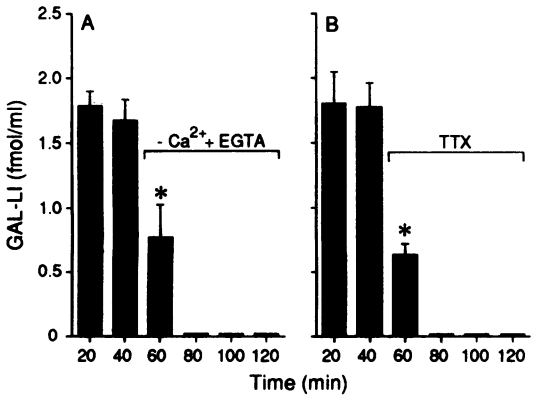

FIG. 2. Ca^{2+} (*A*) and TTX (*B*) dependence of the *in vivo* spontaneous efflux of GAL-LI from rat ventral hippocampus. Animals were perfused at a constant rate of 5 μl/min and perfusate was collected every 20 min. After 40 min (baseline), the probe was infused with Ca^{2+}-free Ringer's solution containing 1 mM EGTA (*A*) or with Ringer's solution containing 1 μM TTX (*B*). Data are mean ± SEM (vertical bars) of six rats, expressed as GAL-LI (fmol/ml) released in 20 min. In *A* and *B*, GAL-LI efflux fell below the detection limit (<0.5 fmol/ml) from the second fraction after Ca^{2+} removal and TTX addition. *, $P < 0.01$ vs. baseline (Dunnett's test).

Table 1. Dose–response effect of veratridine on GAL-LI efflux from ventral hipocampus

Veratridine, μM	GAL-LI eflfux, fmol per ml per 20 min	% of baseline
None	1.87 ± 0.1	100
37	4.41 ± 0.1*	236
74	7.06 ± 0.4*	378
148	11.71 ± 0.4*	626

Veratridine was applied by reverse dialysis for 40 min after an 80-min baseline (no veratridine) into the ventral hippocampus. Data are the mean ± SEM of the peak increase fraction, which occurred 20 min after the start of veratridine infusion, and expressed as GAL-LI content (fmol/ml) in the 20-min fraction. *, $P < 0.01$ vs. baseline (Dunnett's test).

perfusion, and this increase persisted in the next 20-min fraction (Fig. 3*B*). When Ca^{2+} was omitted and 1 mM EGTA was added in the perfusion solution 40 min before potassium stimulation, KCl-induced GAL-LI release was inhibited by 75% on average.

***In Vitro* GAL-LI Release.** The spontaneous efflux of GAL-LI from slices of rat ventral hippocampus was 1.0 ± 0.02 fmol per ml per 5 min (n = 19 sets of slices) and did not significantly change over 40 min (data not shown). Spontaneous outflow

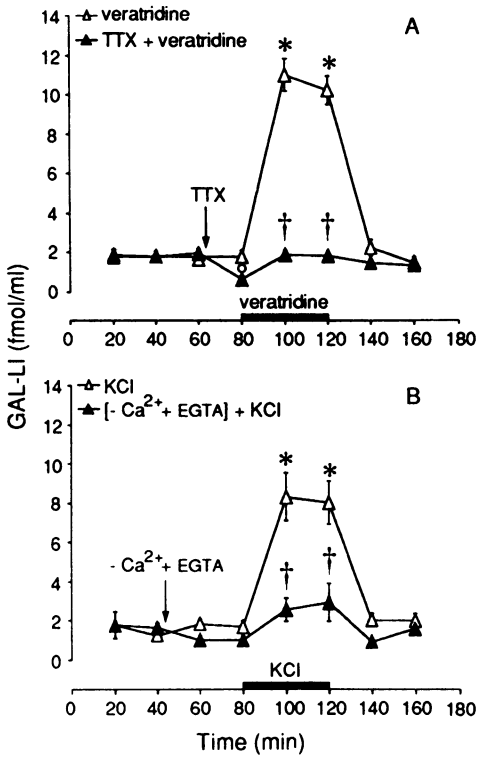

FIG. 3. Effect of 148 μM veratridine (*A*) or 60 mM KCl (*B*) on the extracellular concentration of GAL-LI in the ventral hippocampus and their respective TTX and Ca^{2+} dependence. (*A*) Dialysate was collected at 20-min intervals for 60 min before addition of TTX. Perfusion with Ringer's solution containing 1 μM TTX was started 20 min before veratridine and continued until the end of the experiment. Veratridine was perfused for 40 min. (*B*) Dialysate was collected at 20-min intervals for 40 min (baseline) before removal of Ca^{2+} and addition of 1 mM EGTA. Perfusion with Ca^{2+}-free Ringer's solution containing EGTA was started 40 min before KCl and continued up to the end of the experiment. KCl was perfused for 40 min. Data are mean ± SEM (vertical bars) of six rats, expressed as GAL-LI (fmol/ml) released in 20 min. $P < 0.01$ for TTX vs. baseline; *, $P < 0.01$ vs. baseline (Dunnett's test); †, $P < 0.01$ for veratridine vs. veratridine plus TTX and for 60 mM KCl vs. ($-Ca^{2+}$ + EGTA) plus 60 mM KCl (Split-plot ANOVA followed by Tukey's test for unconfounded means).

was drastically reduced (<0.5 fmol/ml, n = 6 sets of slices) after a 10-min incubation with Ca^{2+}-free Ringer's solution containing 1 mM EGTA (data not shown). KCl enhanced GAL-LI in the release medium in a concentration-dependent manner; 25, 50, and 100 mM KCl induced, respectively, 170, 500, and 850% increases in GAL-LI extracellular concentration during a 10-min incubation (Fig. 4). The effect of 50 mM KCl was suppressed when the slices were incubated in a Ca^{2+}-free Ringer's solution containing 1 mM EGTA for 5 min before and during a 10-min KCl stimulation (5.6 ± 0.6 fmol per ml per 5 min in normal Ringer's solution but <0.5 fmol/ml in Ca^{2+}-free Ringer's solution containing EGTA; n = 6 sets of slices per experimental group).

Diagonal-Band Electrical Stimulation. Electrical stimulation of the ventral limb of the diagonal band induced a frequency-dependent increase in GAL-LI concentration in the perfusate of the ventral hippocampus (Fig. 5). A 10-Hz stimulation had no effect in releasing GAL-LI but 50 Hz and 100 Hz increased GAL-LI efflux by 146 and 158%, respectively. The maximum effect, 230%, was attained at 200 Hz. A second 50-Hz stimulation train delivered to the animals 100 min after the first 50-Hz septal tetanus induced a 139 ± 7% ($P < 0.05$) increase in GAL release above spontaneous efflux (data not shown).

To establish whether this enhancement was of neuronal origin, 5 μM TTX was included in the dialysis probe for 60 min starting 20 min before the electrical stimulation. TTX blocked the effect of 200 Hz on GAL-LI efflux and reduced the spontaneous efflux of GAL-LI to below the level of detection (<0.5 fmol/ml) during a 60-min perfusion (Fig. 6).

DISCUSSION

This study demonstrates the *in vivo* release of the neuropeptide GAL in the rat central nervous system. The release of endogenous GAL was measured in the rat ventral hippocampus by microdialysis coupled with a sensitive RIA. The specificity of the antiserum for this peptide has been proved (23, 24). Sephadex G-50 chromatography or HPLC of rat brain tissue showed that GAL-LI, measured with the same antiserum we used, consists primarily of full-length peptide. Although we did not chromatographically separate GAL-LI in dialysate samples because of the low sensitivity of the HPLC method available (24), we found a parallel between serial dilutions of dialysate of ventral hippocampi and synthetic GAL standards in displacing the specific tracer binding to the antiserum. This makes it unlikely that endogenous factors

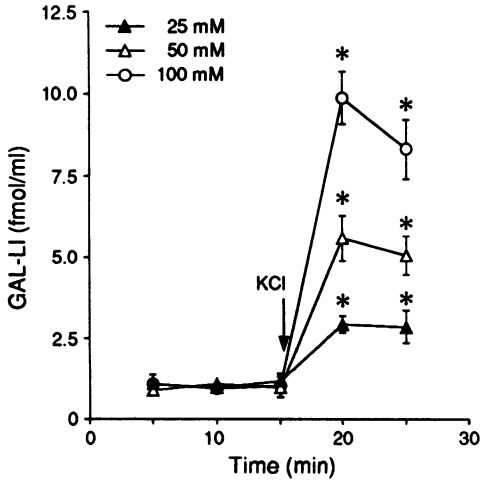

FIG. 4. Effect of various concentrations of KCl on GAL-LI efflux from slices of ventral hippocampus. Samples were collected every 5 min for 15 min (baseline) before addition of KCl to the medium. Data are mean ± SEM (vertical bars) of two experiments in triplicate and are expressed as GAL-LI (fmol/ml) released in 5 min. *, $P < 0.01$ vs. baseline (Dunnett's test).

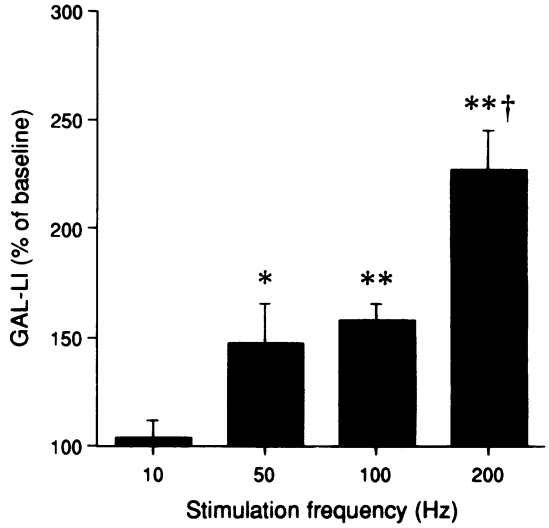

FIG. 5. Frequency-dependent effect of electrical stimulation of the ventral limb of the diagonal band on GAL-LI efflux from ventral hippocampus. Electrical stimuli (10, 50, 100, and 200 Hz) were delivered bilaterally (20 repeated at 2-min intervals). Each rat was implanted in both ventral hippocampi. The dialysate was collected from each hippocampus at 20-min intervals for 40 min (baseline), before electrical stimulation. GAL-LI did not differ in bilateral samples (<10%) at any time point before and during electrical stimulation. Data are mean ± SEM (vertical bars) of 10 hippocampi from five rats. The results are expressed as the percentage of baseline, which was 1.89 ± 0.3 fmol/ml in 20 min. Statistical analysis was done on absolute values. The effect of a 200-Hz electrical stimulation was significantly greater (†, $P < 0.01$) than the effect of 10, 50, or 100 Hz (Duncan's test). *, $P < 0.05$; **, $P < 0.01$ vs. respective baseline (Dunnett's test).

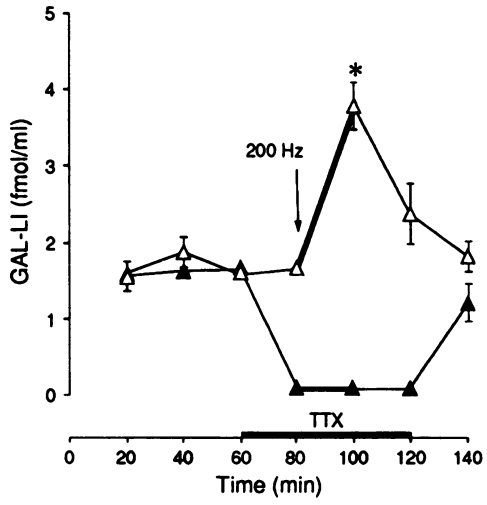

FIG. 6. Effect of a 200-Hz electrical stimulation of the ventral limb of the diagonal band on GAL-LI efflux from the ventral hippocampus and its TTX-dependence. The dialysate was collected (baseline) at 20-min intervals for 60 min before addition of TTX. Perfusion with Ringer's solution containing 5 μM TTX was started 20 min before electrical stimulation and continued until the end of the experiment. Electrical stimulation (200 Hz) was delivered bilaterally for 20 min (140 μA, 10 0.5-ms 10-V pulses applied for 10 s and repeated at 2-min intervals). Data are mean ± SEM (vertical bars) GAL-LI (fmol/ml) concentrations in 20-min fractions collected from 10 hippocampi of five rats. Baseline values and the electrical stimulation effect did not differ in the hippocampi of both hemispheres. △, Electrical stimulation; ▲, electrical stimulation plus TTX. *, $P <$ 0.01 vs. baseline (Dunnett's test).

other than GAL could interfere with the RIA. Furthermore, extracellular GAL-LI was not detectable in cerebellar tissue, which immunocytochemistry and *in situ* hybridization analysis has shown is devoid of GAL (25).

The *in vivo* GAL release appears to satisfy the criteria for physiological release. Basal output of GAL-LI from ventral hippocampus was Ca^{2+}-dependent and TTX-sensitive and, therefore, depended on action potential propagation. Opening voltage-dependent Na^+ channels with veratridine or K^+ depolarization caused significant enhancement of GAL-LI release, the effects being TTX-sensitive and Ca^{2+}-dependent, respectively. *In vitro* evidence indicates that GAL-LI is released from ventral hippocampal slices in a Ca^{2+}- and depolarization-dependent manner. These results suggest that GAL is released from a neuronal compartment in the hippocampus in an impulse flow-dependent manner and it may function there as a neurotransmitter.

There are many sources of GAL-LI release in the ventral hippocampus, since the peptide is found in several types of afferent and local neurons (1, 2). The present study shows that the hippocampal GAL-LI outflow is related to the activity of the cholinergic GAL-LI-containing cells in the septal/diagonal band complex. Thus, electrical field stimulation of the ventral limb of the diagonal-band nuclei, where virtually all GAL-LI-positive cells contain choline acetyltransferase-like immunoreactivity (26, 27), caused a frequency-dependent overflow of GAL-LI.

The stimulation frequencies required to release measurable amounts of GAL-LI in the hippocampus were 50 Hz and above, which are higher than those required to increase AcCho release *in vivo* from the septal/diagonal-band neurons (28). This is in line with evidence that in most of the peptidergic and classical neurotransmitter-containing neurons, peptide release requires higher stimulation frequencies or other patterns of stimuli than the classical neurotransmitters (29–31). The higher frequency threshold for the release of GAL relative to AcCho has physiological significance and implies that at low stimulation frequencies the neurons containing AcCho and GAL selectively release AcCho, which is under muscarinic autoreceptor control (32, 33). At higher impulse frequencies, when GAL is also released, other mechanisms may come into play to counteract excessive cholinergic activity. This means GAL would activate presynaptic mechanisms to exert inhibitory control over AcCho release (9), while at the same time the released GAL would act postsynaptically to inhibit the muscarinic stimulation of inositol phospholipid turnover (14).

These mechanisms could play a role in the neuropathology of Alzheimer disease, where the population of cholinergic and thus cholinergic/galaninergic forebrain neurons is declining and the surviving neurons increase their firing rate, as suggested by AcCho turnover studies (34). Under these conditions, the activity of GAL as a regulator of the synaptic concentration of AcCho might be expected to increase greatly. Interestingly, there is evidence that the concentration of GAL is increased in the cerebral cortex (35) and in the basal forebrain of Alzheimer disease patients (34).

In summary, endogenous GAL appears to be a putative neurotransmitter released from the cholinergic/galaninergic septal hippocampal neurons. GAL-mediated inhibition of the pre- and postsynaptic actions of AcCho is, therefore, a likely part of the overall regulation of cholinergic transmission in the hippocampus. This situation and the demonstration of GAL receptors and release make hippocampal galaninergic transmission an interesting target for attempts to enhance cholinergic neurotransmission in pathological conditions such as senile dementia of the Alzheimer type.

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