Electrophysiological evidence for a hyperpolarizing, galanin (1-15)-selective receptor on hippocampal CA3 pyramidal neurons

Zhi-Qing David Xu*†, Xiaosong Ma*, Ursel Soomets‡, Ulo Langel‡, and Tomas Hökfelt*

*Department of Neuroscience, Karolinska Institute, and ‡Department of Neurochemistry and Neurotoxicology, Stockholm University, S-171 77 Stockholm, Sweden

Contributed by Tomas Hökfelt, October 21, 1999

The effects of the 29-amino acid neuropeptide galanin [GAL (1–29)], GAL(1–15), GAL(1–16), and the GAL subtype 2 receptor agonist D-tryptophan^2-GAL(1–29) were studied in the dorsal hippocampus *in vitro* with intracellular recording techniques. GAL(1–15) induced, in the presence of tetrodotoxin, a dose-dependent hyperpolarization in hippocampal CA3 neurons. Most of the GAL(1–15)-sensitive neurons did not respond to GAL(1–29), GAL(1–16), or D-tryptophan^2-GAL(1–29). These results indicate the presence of a distinct, yet-to-be cloned GAL(1–15)-selective receptor on CA3 neurons in the dorsal hippocampus.

coexistence | locus coeruleus | neuropeptide | noradrenaline

Galanin (GAL), a 29-amino acid peptide originally isolated from porcine intestine (1), has a widespread distribution in the central nervous system, as shown with both histochemical and biochemical techniques (2–5). The sequence of GAL is known in many species and has a remarkable and complete homology at the N-terminal moiety, GAL(1–15) (reviewed in ref. 6). Structure activity studies have shown that the N-terminal portion of GAL is important for recognition by the receptor in the central nervous system (7, 8). Recently, cDNAs for three distinct GAL receptors (GALR) have been identified, the GALR1 (9–11), GALR2 (12–14), and GALR3 (15) receptors, and they belong to the superfamily of seven transmembrane, G-protein-linked receptors (reviewed in refs. 16 and 17). Activation of GALRs results in opening of K^+ channels, inhibition of cAMP synthesis (GALR1 and GALR3; refs. 15 and 18), and probably activation of phospholipase C (GALR2; ref. 13).

It has been shown that GAL has various effects at the hippocampal level by using neurochemical and behavioral analyses (reviewed in refs. 19 and 20). Most endogenous GAL in the hippocampus and dentate gyrus of normal rats is present in noradrenergic fibers (21–23). Moreover, in the dorsal hippocampus, GALR2 and GALR3 receptor mRNAs have been observed in granule and pyramidal cells (21, 24–26). However, by using ^{125}I-GAL(1–29) as ligand in autoradiographic studies on rat brain, a distinct binding was found only in the ventral hippocampus and not over dorsal cortical and hippocampal areas (27–29). In agreement, it has been reported that GAL(1–29) does not change the membrane potential when recording intracellularly from hippocampal CA1 neurons (30). Another electrophysiological study indicates that GAL(1–29) inhibits the expression of long-term potentiation without any changes in the size of the evoked synaptic potentials in CA1 neurons in guinea pig hippocampal slice preparations (31). Interestingly, Hedlund *et al.* (32) have shown strong binding in cortex and the dorsal hippocampus (and striatum) by using ^{125}I-GAL(1–15)-ol as ligand. In agreement, functional studies show that GAL(1–15), but not GAL(1–29), has effects in the dorsal hippocampus, suggesting the presence of GALR subtypes (33).

The functional significance of GAL binding sites that preferentially interact with the truncated peptide GAL(1–15) is not clear, and their relationship to GALRs in the dorsal hippocampus remains to be defined. In the present study, the effects of the full-length peptide GAL(1–29), of the two GAL fragments GAL(1–15) and GAL(1–16), and of the GALR2 "agonist" D-Trp^2-GAL(1–29) (Trp, tryptophan; ref. 13) were studied in the dorsal hippocampus. For comparison, locus coeruleus (LC) neurons were also investigated.

Methods

Male Sprague–Dawley rats (200–300 g; Alab, Stockholm) were used in the experiments. Each rat was decapitated, and the brain was excised rapidly over a cold plate. Transverse dorsal hippocampal slices (300- to 400-μm thick) were prepared with a Vibratome (Ted Pella, Redding, CA) in ice-cold artificial cerebrospinal fluid (ACSF), saturated with 95% O_2/5% CO_2. The ACSF contained (in mM) 124 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 1.24 NaH_2PO_4, 2.4 $CaCl_2$, 25 $NaHCO_3$, and 10 glucose. Horizontal slices including LC were prepared as described above and elsewhere (34). The LC could be easily identified in the transilluminated slice as a dark oval area on the lateral edge of the fourth ventricle. Slices were then transferred to a submerged chamber perfused with oxygenated ACSF at 35–37°C.

Conventional intracellular recordings were made from dorsal hippocampal CA3 pyramidal neurons and LC neurons with sharp microelectrodes filled with 2 M potassium chloride (dc resistance between 55 and 80 MΩ). Neurons were routinely held near their resting membrane potential.

GAL(1–29), GAL(1–16), and D-Trp^2-GAL(1–29), a GAL-R2 agonist, were from Bachem. GAL(1–15) was synthesized as C-terminally free carboxylic acid as described (35). Tetrodotoxin (TTX) was from Sigma. GAL(1–15), GAL(1–16), and GAL(1–29) were dissolved in ACSF as stock solution (10^{-3} M, calculated on the basis of purity of the peptide) and stored at −70°C in aliquot vials. Drugs were dissolved in warmed, oxygenated ACSF just before use and applied either via bath perfusion or by turning a three-way valve that switched from ACSF to the test solution. All data were stored on a PC for on-line and off-line analysis with AXOTAPE, AXOGRAPH, and PCLAMP software (all from Axon Instruments, Foster City, CA). All data are from preparations that showed significant recovery on washout (except where indicated). The data are expressed as means ± SEM. Statistical comparisons were performed by using Student's *t* test, and statistical differences were considered significant at $P \leq 0.05$.

Results

Effects on Hippocampal CA3 Neurons. Bath application of GAL(1–15) (10–1,000 nM) evoked a reversible membrane hyperpolar-

Abbreviations: ACSF, artificial cerebrospinal fluid; Gal, galanin; GALR*n*, GAL receptor subtype *n*; LC, locus coeruleus; Trp, tryptophan; TTX, tetrodotoxin.

†To whom reprint requests should be addressed at: Department of Neuroscience, Karolinska Institutet; S-171 77 Stockholm, Sweden.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

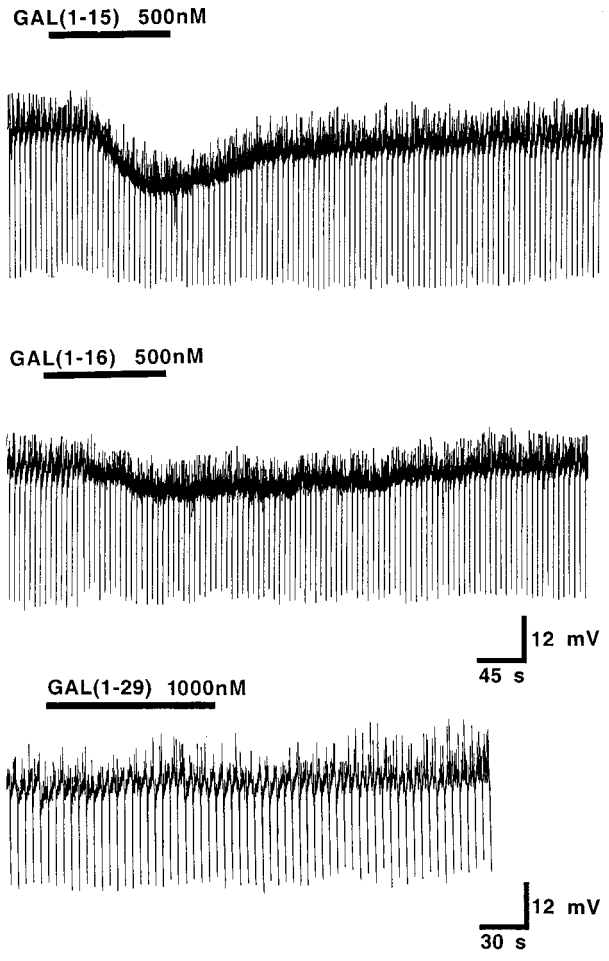

Fig. 1. GAL(1–15)-induced hyperpolarization of a CA3 neuron. Bath application of GAL(1–15) induces hyperpolarization, accompanied by a slight decrease in membrane resistance (*Top*), and the same concentration of GAL(1–16) induces hyperpolarization with a smaller amplitude (*Middle*), whereas GAL(1–29) does not have any effects on the same neuron, even with a higher concentration (*Bottom*). The resting potential was −68 mV.

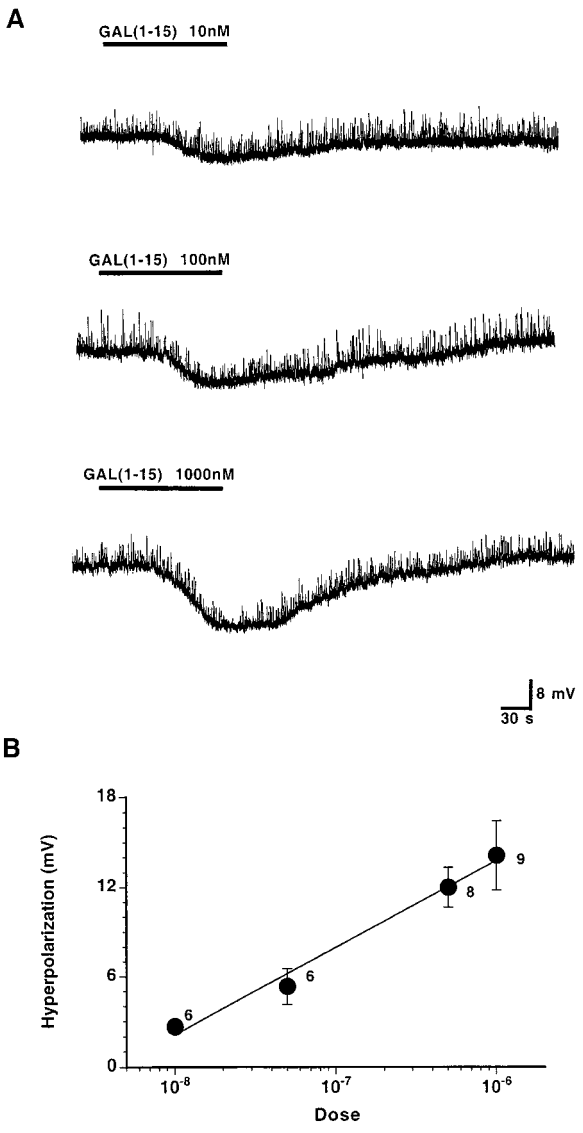

Fig. 2. (*A*) Dose-dependent GAL(1–15)-induced hyperpolarization. The resting potential was −70 mV. (*B*) Dose-response curve for CA3 neurons to GAL(1–15). The amplitude of the hyperpolarization was plotted as a function of the logarithmic concentration of GAL(1–15) applied by superfusion.

ization (1–20 mV) (Fig. 1) and inhibition of spike discharge in 24 of 51 tested neurons (Table 1). When tested on individual neurons, the effects of GAL(1–15) were dose-dependent (Fig. 2 *A* and *B*). The time required for full recovery was variable (2–15 min; see Figs. 1–4), suggesting involvement of a long-lived second messenger or messengers in mediating the effects of GAL. Based on changes in the size of the voltage responses seen after brief (30-ms), repetitive hyperpolarizing current pulses of constant amplitude, the hyperpolarizing responses to GAL(1–15) ($n = 12$) were accompanied by increases in mean membrane conductance (23 ± 5%; Fig. 1). This change in input conductance was not attributable to the membrane hyperpolarization alone, because a decrease in input resistance still occurred when the membrane potential was held constant during the application of GAL(1–15) ($n = 5$). The effect was identical, regardless of whether the intracellular electrode contained acetate or chloride anions. Hyperpolarizing responses and increases in membrane

Table 1. Hyperpolarizing effect of GAL and GAL fragments/analog on hippocampal CA3 and LC neurons

	Hippocampal CA3, no. of neurons			LC, no. neurons		
GAL fragment/analog	Response	No response	Total	Response	No response	Total
GAL(1–15)	24	27	51	31	5	36
GAL(1–16)	6	18	24	30	0	30
GAL(1–29)	6	36	40	52	0	52
D-Trp^2-GAL(1–29)	5	17	22	—	—	—

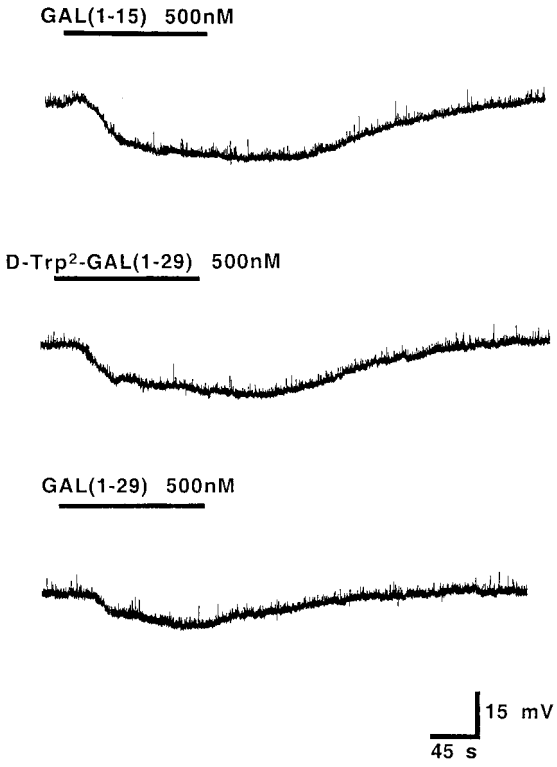

Fig. 3. D-Trp2-GAL(1–29)- and GAL(1–29)-induced hyperpolarization of a GAL(1–15)-sensitive CA3 neuron. GAL(1–15) induces hyperpolarization (*Top*). The same concentration of D-Trp2-GAL(1–29) (*Middle*) as well as GAL(1–29) (*Bottom*) induces a hyperpolarization with smaller amplitude on the same neuron. The resting potential was −71 mV.

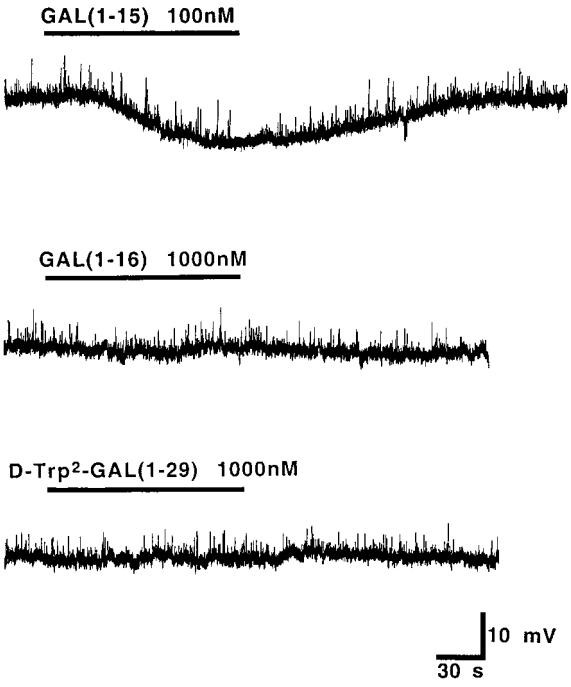

Fig. 4. Action of GAL(1–16) and D-Trp2-GAL(1–29) on a GAL(1–15)-sensitive CA3 neuron. Bath application of GAL(1–15) induces hyperpolarization (*Top*), but neither GAL(1–16) (*Middle*) nor D-Trp2-GAL(1–29) (*Bottom*) has an effect on the same neuron, even at a higher concentration. The resting potential was −70 mV.

conductance induced by GAL(1–15) were retained in the presence of 1 μM TTX (n = 4), suggesting that the receptors mediating these effects are expressed on the plasma membrane of CA3 neurons.

Application of GAL(1–16) (Fig. 1), GAL(1–29) (Fig. 3), or the GALR2-preferring analog D-Trp^2-GAL(1–29) (Fig. 3) also evoked a reversible membrane hyperpolarization and inhibition of spike discharge in some of the tested neurons. Thus, GAL(1–16) hyperpolarized 6 of 24 tested neurons (Table 1). GAL(1–29) caused hyperpolarization but in fewer neurons (6 of 40) (Table 1), and the effect was weaker (Fig. 3). D-Trp^2-GAL(1–29) affected 5 of 22 tested neurons (Table 1). All GAL(1–16)-, GAL(1–29)-, and D-Trp^2-GAL(1–29)-sensitive neurons responded to GAL(1–15) (Figs. 1 and 3). However, most of the GAL(1–15)-sensitive neurons did not respond to GAL(1–29) (Fig. 1), GAL(1–16), or D-Trp^2-GAL(1–29) (Fig. 4). Thus, GAL(1–29) induced a hyperpolarization only in 6 of 16 GAL(1–15)-sensitive neurons (Fig. 3). GAL(1–16) hyperpolarized 6 of 14 GAL(1–15)-sensitive neurons (Fig. 1), whereas D-Trp^2-GAL(1–29) affected 5 of 14 GAL(1–15)-sensitive neurons (Fig. 3). GAL(1–16) caused a hyperpolarization in all four tested GAL(1–29)-sensitive neurons. However, GAL(1–29) affected three GAL(1–16)-sensitive neurons but not the other two (Fig. 1). GAL(1–29), GAL(1–16), and D-Trp^2-GAL(1–29) had no effect on those neurons that did not respond to GAL(1–15).

Effects on LC Neurons. Bath applications of GAL(1–15) inhibited the spontaneous firing and hyperpolarized 32 of 36 tested LC neurons (Fig. 5). The GAL(1–15)-induced hyperpolarization was fully reversible and lasted 1–8 min in most neurons. GAL(1–15) induced hyperpolarization in LC neurons exposed to ACSF containing TTX (n = 4).

Bath application of GAL(1–29) induced hyperpolarization in all GAL(1–15)-tested neurons (n = 36) as described (36), and also GAL(1–16) caused hyperpolarization in all 36 GAL(1–15)-tested neurons. The response of all these three compounds was dose-dependent (Fig. 5). However, in contrast to the results obtained in the dorsal hippocampus, GAL(1–16) and GAL(1–29) even more so had, on a molar basis, a stronger effect than GAL(1–15).

Discussion

The present results provide electrophysiological evidence for a distinct, yet-to-be cloned GAL-like receptor that is GAL(1–15) selective. Thus, GAL(1–15), in the presence of TTX, induces hyperpolarization in dorsal hippocampal CA3 neurons, suggesting the presence of a postsynaptic GALR on these pyramidal cells. This finding is in agreement with previous autoradiographic and pharmacological/functional studies describing binding and effects of GAL(1–15)-ol/GAL(1–15) in the hippocampus (32, 33).

The effects of GAL on the membrane properties of hippocampal neurons were first studied by Dutar *et al.* (30), who reported that bath application of GAL(1–29) caused only a slight hyperpolarization of CA1 neurons in the ventral hippocampus without an obvious change in membrane resistance. However, GAL(1–29) strongly reduced an atropine-sensitive, long-lasting depolarization evoked by train stimulation (30). These results indicate a presynaptic action of GAL on acetylcholine-releasing nerve terminals synapsing on CA1 neurons. The results agree with autoradiographic studies that suggest a presynaptic localization of GAL(1–29) binding sites in the ventral hippocampus and with the demonstration of GAL(1–29)-induced inhibition of acetylcholine release (37). It has also been reported that GAL specifically blocks the expression of long-term potentiation in CA1 neurons in guinea pig hippocampal slices (31). Moreover,

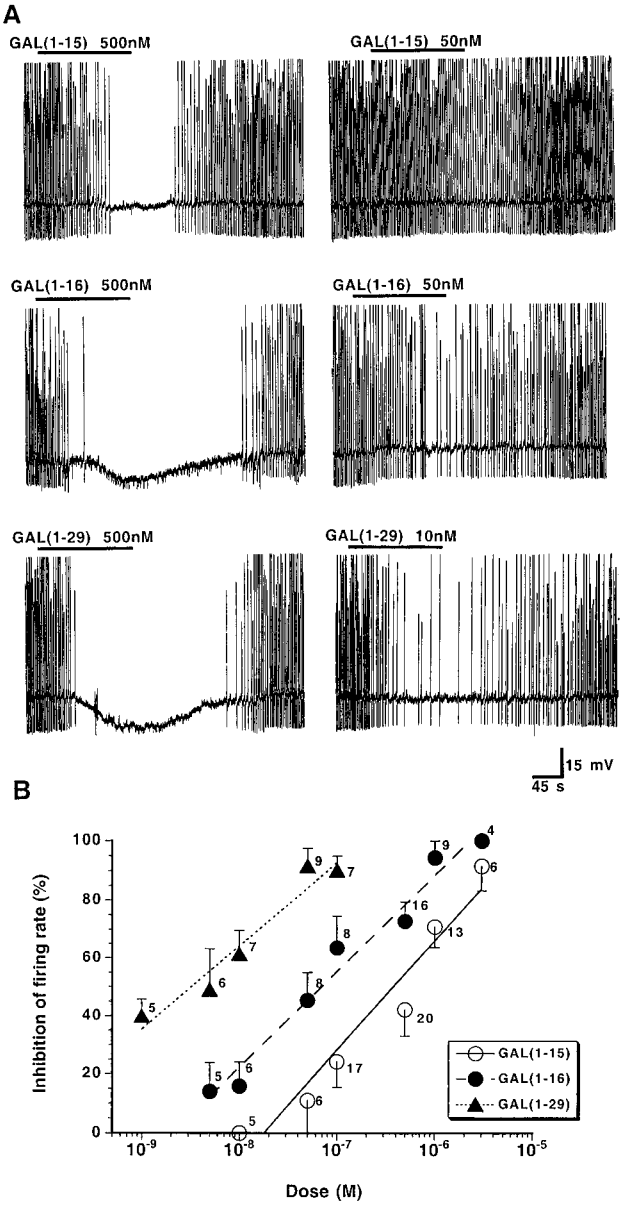

Fig. 5. (*A*) Dose-response effect of GAL(1–15), GAL(1–16), and GAL(1–29) on the same LC neuron. Note that GAL(1–15) (*Top*) has a distinctly weaker effect than GAL(1–16) (*Middle*) and GAL(1–29) (*Bottom*), causing hardly any hyperpolarization (*Top Right*). The resting potential was −60 mV throughout. (*B*) Dose-response curves for LC neurons to GAL(1–15) (open circles), GAL(1–16) (filled circles), and GAL(1–29) (filled triangles). The order of sensitivity is GAL(1–29) > GAL(1–16) > GAL(1–15).

several behavioral studies that used various *in vivo* models have shown inhibitory effects of GAL in memory and learning tests (reviewed in refs. 19 and 20).

In the present study, GAL(1–29) and two GAL fragments, GAL(1–15) and GAL(1–16), were tested on dorsal hippocampal CA3 pyramidal cells, whereby many neurons responded to GAL(1–15) but fewer responded to GAL(1–16) and GAL(1–29). A distinct, TTX-resistant hyperpolarization was observed, suggesting that GAL(1–15) has a direct effect on CA3 neurons via a postsynaptic GALR. It has been reported that there is a binding site for GAL(1–15)-ol in the dorsal hippocampus (and in cortex and striatum; ref. 32), regions where very little GAL(1–29) binding could be detected (27–29). Moreover, GAL(1–15), but not GAL(1–29), produces an increase in the K_D value of the 5-HT_{1A} agonist (^{3}H)8-OH-2-(di-*n*-propylamino)-tetralin, thus reducing the affinity of 5-HT_{1A} receptors (33). This effect in the dorsal hippocampus does not seem to be related to inhibition of adenylyl cyclase (38), in contrast to the effect of GAL(1–29) and GAL(1–15) in other brain regions (38–41). Taken together, the results suggest that there is a cortical/hippocampal GAL(1–15)-selective receptor that does not bind GAL(1–29). Such a receptor may also be present in the periphery (42–45). A previous study indicated that the potency of GAL analogs decreases substantially after the removal of the histidine in position 16 (46). On the other hand, as shown here and in other studies (33, 38, 42–45), removal of this amino acid substantially increases potency in the dorsal hippocampal CA3 neuron preparation. Thus, position 16 in GAL(1–29) may be critical for the GAL subtype receptors.

Until now, three GALR subtypes, GALR1 (9–11), GALR2 (12–14), and GALR3 (15), have been cloned. *In situ* hybridization studies have reported that GALR2 and GALR3 mRNAs are expressed in the hippocampal CA1–CA3 fields and the granule cell layer (21, 24–26), indicating a possible presence of postsynaptically located GALRs in the two major cell systems in the hippocampal formation. However, binding studies on GALRs expressed in cell lines show that all three subtypes of GALRs, including GALR1, which has not been found in the dorsal hippocampus (11, 26, 47), bind GAL(1–29) with high affinity (18, 48). In the present study, it is striking that we observed a subpopulation of cells that responded only to GAL(1–15) but not to GAL(1–29) or GAL(1–16). Moreover, D-Trp^2-GAL(1–29), a GALR2 agonist (13), did not have any effect on some GAL(1–15)-sensitive neurons. This result suggests that the GAL(1–15)-selective binding site is unlikely to be identical to any of the three cloned receptors but rather represents a distinct GALR subtype. It should be noted that GAL(1–29) and GAL(1–16) also induced hyperpolarization in CA3 neurons, albeit in fewer neurons, and that these neurons also responded to GAL(1–15). This result indicates a possible overlap in function of multiple receptors and the possibility that several GALRs may be colocalized in certain CA3 neurons.

The inhibitory actions of GAL(1–15) were identical whether or not the intracellular electrode contained chloride ions. The effects of this peptide, therefore, presumably do not involve chloride-permeable channels. Because hyperpolarizing responses were associated with an increase in membrane conductance, it is likely that a component of the GAL-mediated hyperpolarization resulted from activation of K^+ channels. GAL has previously been reported to increase K^+ conductance in central neurons (21, 36, 49, 50) as well as in endocrine cells (51, 52) and autonomic neurons (53). The nature of the K^+ conductance presumably involved in mediating the effects of GAL(1–15) in hippocampus remains to be established.

It has been reported that GAL induces a decrease in firing rate and a hyperpolarization of LC neurons in slice preparations (11, 49, 54, 55). In the present study, GAL(1–15), GAL(1–16), and GAL(1–29) induced hyperpolarization of LC neurons. Expression of mRNAs of both GALR1 and GALR2 in LC neurons has been reported (11, 26). In contrast to the results in the hippocampus, GAL(1–16) and GAL(1–29) affected all tested LC neurons, whereas GAL(1–15) caused a hyperpolarization in 31 of 36 tested neurons and had the weakest effect among the tested peptides. These data show that the GALR or GALRs present in LC neurons bind GAL(1–29) with a higher affinity than GAL(1–15) and that some LC neurons respond only to GAL(1–29) and GAL(1–16) and not GAL(1–15). The putative distinct receptor subtype sensitive to GAL(1–15) may therefore not be expressed in LC neurons.

GAL coexists with noradrenaline in a large proportion (about 80%) of the neurons in the LC (56, 57). We have proposed that GAL(1–29) released from LC cell somata/dendrites may act on

postsynaptic (auto)receptors, which bind GAL(1–29) with high affinity (21, 36). A previous study has shown that, in the dorsal hippocampal formation, GAL is present in detectable concentrations in noradrenergic nerve endings originating in LC (21). To what extent GAL(1–29) can be processed to GAL(1–15) inside or outside the GAL/noradrenaline terminals in the dorsal hippocampus to act on a putative GAL(1–15) receptor on CA3 pyramidal neurons remains to be determined. Previous work has shown that prolonged burst-type stimulation of the locus coeruleus, which should particularly effectively release coexisting neuropeptides (58), causes a prolonged, reserpine-resistant inhibition of midbrain dopamine neurons with noradrenergic input (59). Such an inhibitory effect, herein proposed to be mediated via galanin released from noradrenaline terminals in the hippocampus (21) under profound stress exposure associated with locus coeruleus activation (reviewed in ref. 60), could hypothetically lead to memory impairment.

We thank Prof. T. Svensson for valuable comments. This study was supported by Marianne and Marcus Wallenberg's Foundation, Swedish Medical Research Council Grant 04X-2887, and a Bristol-Myers Squibb Unrestricted Neuroscience Grant. Z.-Q.D.X. was supported by Hjärnfonden and the Wenner-Gren Foundations, and X.M. was supported by the Swedish Institute.

1. Tatemoto, K., Rökaeus, A., Jörnvall, H., McDonald, T. J. & Mutt, V. (1983) *FEBS Lett.* **164,** 124–128.
2. Rökaeus, A., Melander, T., Hökfelt, T., Lundberg, J. M., Tatemoto, K., Carlquist, M. & Mutt, V. (1984) *Neurosci. Lett.* **47,** 161–166.
3. Skofitsch, G. & Jacobowitz, D. M. (1985) *Peptides* **6,** 509–546.
4. Skofitsch, G. & Jacobowitz, D. M. (1986) *Peptides* **7,** 609–613.
5. Melander, T., Hökfelt, T. & Rökaeus, A. (1986) *J. Comp. Neurol.* **248,** 475–517.
6. Langel, Ü. & Bartfai, T. (1998) *Ann. N.Y. Acad. Sci.* **863,** 86–93.
7. Fisone, G., Berthold, M., Bedecs, K., Undén, A., Bartfai, T., Bertorelli, R., Consolo, S., Crawley, J., Martin, B., Nilsson, S., *et al*. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 9588–9591.
8. Fisone, G., Langel, U., Carlquist, M., Bergman, T., Consolo, S., Hökfelt, T., Undén, A., Andell, S. & Bartfai, T. (1989) *Eur. J. Biochem.* **181,** 269–276.
9. Habert-Ortoli, E., Amiranoff, B., Loquet, I., Laburthe, M. & Mayaux, J. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 9780–9783.
10. Burgevin, M. C., Loquet, I., Quarteronet, D. & Habert-Ortoli, E. (1995) *J. Mol. Neurosci.* **6,** 33–41.
11. Parker, E. M., Izzarelli, D. G., Nowak, H. P., Mahle, C. D., Iben, L. G., Wang, J. & Goldstein, M. E. (1995) *Mol. Brain Res.* **34,** 179–189.
12. Howard, A. D., Tan, C., Shiao, L. L., Palyha, O. C., Mckee, K. K., Weinberg, D. H., Feighner, S. D., Cascieri, M. A., Smith, R. G., Vanderploeg, L., et al. (1997) *FEBS Lett.* **405,** 285–290.
13. Smith, K. E., Forray, C., Walker, M. W., Jones, K. A., Tamm, J. A., Bard, J., Branchek, T. A., Linemeyer, D. L. & Gerald, C. (1997) *J. Biol. Chem.* **272,** 24612–24616.
14. Wang, S., He, C., Maguire, M., Clemmons, A., Burrier, R., Guzzi, M., Strader, C., Parker, E. & Bayne, M. (1997) *FEBS Lett.* **411,** 225–230.
15. Wang, S., He, C., Hashemi, T. & Bayne, M. (1997) *J. Biol. Chem.* **272,** 31949–31952.
16. Iismaa, T. P., Fathi, Z., Hort, Y. J., Iben, L. G., Dutton, J. L., Baker, E., Sutherland, G. R. & Shine, J. (1998) *Ann. N.Y. Acad. Sci.* **863,** 56–63.
17. Branchek, T., Smith, K. E. & Walker, M. W. (1998) *Ann. N.Y. Acad. Sci.* **863,** 94–107.
18. Smith, K., Walker, M., Artymyshyn, R., Bard, J., Borowsky, B., Tamm, J., Yao, W., Vaysse, P., Branchek, T., Gerald, C., *et al*. (1998) *J. Biol. Chem.* **273,** 23321–23326.
19. McDonald, M. P., Gleason, T. C., Robinson, J. K. & Crawley, J. N. (1998) *Ann. N.Y. Acad. Sci.* **863,** 305–322.
20. Ögren, S. O., Schött, P., Kehr, J., Yoshitake, T., Misane, I., Mannström, P. & Sandin, J. (1998) *Ann. N.Y. Acad. Sci.* **863,** 342–363.
21. Xu, Z.-Q. D., Shi, T.-J. S. & Hökfelt, T. (1998) *J. Comp. Neurol.* **392,** 227–252.
22. Melander, T., Staines, W. A. & Rökaeus, A. (1986) *Neuroscience* **19,** 223–240.
23. Gabriel, S. M., Knott, P. J. & Haroutunian, V. (1995) *J. Neurosci.* **15,** 5526–5534.
24. Fathi, Z., Cunningham, A. M., Iben, L. G., Battaglino, P. B., Ward, S. A., Nichol, K. A., Pine, K. A., Wang, J., Goldstein, M. E., Iismaa, T. P., *et al*. (1997) *Mol. Brain Res.* **51,** 49–59.
25. Kolakowski, L. J., O'Neill, G., Howard, A., Broussard, S., Sullivan, K., Feighner, S., Sawzdargo, M., Nguyen, T., Kargman, S., Shiao, L., *et al*. (1998) *J. Neurochem.* **71,** 2239–2251.
26. O'Donnell, D., Ahmad, S., Wahlestedt, C. & Walker, P. (1999) *J. Comp. Neurol.* **409,** 469–481.
27. Skofitsch, G., Sills, M. A. & Jacobowitz, D. M. (1986) *Peptides* **7,** 1029–1042.
28. Melander, T., Hökfelt, T., Nilsson, S. & Brodin, E. (1986) *Eur. J. Pharmacol.* **124,** 381–382.
29. Melander, T., Köhler, C., Nilsson, S., Hökfelt, T., Brodin, E., Theodorsson, E. & Bartfai, T. (1988) *J. Chem. Neuroanat.* **1,** 213–233.
30. Dutar, P., Lamour, Y. & Nicoll, R. A. (1989) *Eur. J. Pharmacol.* **164,** 355–360.
31. Sakurai, E., Maeda, T., Kaneko, S., Akaike, A. & Satoh, M. (1996) *Neurosci. Lett.* **212,** 21–24.
32. Hedlund, P. B., Yanaihara, N. & Fuxe, K. (1992) *Eur. J. Pharmacol.* **224,** 203–205.
33. Hedlund, P. B., Finnman, U. B., Yanaihara, N. & Fuxe, K. (1994) *Brain Res.* **634,** 163–167.
34. Xu, Z. Q., Pieribone, V. A., Zhang, X., Grillner, S. & Hökfelt, T. (1994) *Exp. Brain Res.* **98,** 75–83.
35. Langel, U., Land, T. & Bartfai, T. (1992) *Int. J. Pept. Protein Res.* **39,** 516–522.
36. Pieribone, V., Xu, Z.-Q., Zhang, X., Grillner, S., Bartfai, T. & Hökfelt, T. (1995) *Neuroscience* **64,** 861–874.
37. Fisone, G., Wu, C. F., Consolo, S., Nordström, O., Brynne, N., Bartfai, T., Melander, T. & Hökfelt, T. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7339–7343.
38. Billecocq, A., Hedlund, P. B., Bolanos-Jiménez, F. & Fillion, G. (1994) *Eur. J. Pharmacol.* **269,** 209–217.
39. Nishibori, M., Oishi, R., Itoh, Y. & Saeki, K. (1988) *J. Neurochem.* **51,** 1953–1955.
40. Chen, Y., Laburthe, M. & Amiranoff, B. (1992) *Peptides* **13,** 339–341.
41. Karelson, E., Laasik, J. & Sillard, R. (1995) *Neuropeptides* **28,** 21–28.
42. Amiranoff, B., Lorinet, A. M., Yanaihara, N. & Laburthe, M. (1989) *Eur. J. Pharmacol.* **163**, 205–207.
43. Ekblad, E., Håkanson, R., Sundler, F. & Wahlestedt, C. (1985) *Br. J. Pharmacol.* **86,** 241–246.
44. Fox, J. E. T., Brooks, B., McDonald, T. J., Barnett, W., Kostolanska, F., Yanaihara, C., Yanaihara, N. & Rökaeus, Å. (1988) *Peptides* **9,** 1183–1189.
45. Yanaihara, N., Mochizuki, T., Iguchi, K., Hoshino, M., Nagashima, T., Takatsuka, N., Ishikawa, J., Greely, G. H. J., Yanaihara, C. & Kuwahara, A. (1991) in *Galanin: A New Functional Multifunctional Peptide in the Neuro-Endocrine System*, eds. Hökfelt, T., Bartfai, T., Jacobowitz, D. & Ottoson, D. (Macmillan, London), pp. 185–196.
46. Lagny-Pourmir, I., Lorinet, A. M., Yanaihara, N. & Laburthe, M. (1989) *Peptides* **10,** 757–761.
47. Gustafson, E. L., Smith, K. E., Durkin, M. M., Gerald, C. & Branchek, T. A. (1996) *NeuroReport* **7,** 953–957.
48. Pang, L., Hashemi, T., Lee, H.-J. J., Maguire, M., Graziano, M. P., Bayne, M., Hawes, B., Wong, G. & Wang, S. (1998) *J. Neurochem.* **71,** 2252–2259.
49. Sevcik, J., Finta, E. P. & Illes, P. (1993) *Eur. J. Pharmacol.* **230,** 223–230.
50. Papas, S. & Bourque, C. W. (1997) *J. Neurosci.* **17,** 6048–6056.
51. de Weille, J., Schmid, A. H., Fosset, M. & Lazdunski, M. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 1312–1316.
52. Dunne, M. J., Bullett, M. J., Li, G. D., Wollheim, C. B. & Petersen, O. H. (1989) *EMBO J.* **8,** 413–420.
53. Konopka, L. M., McKeon, T. W. & Parsons, R. L. (1989) *J. Physiol. (London)* **410,** 107–122.
54. Seutin, V., Verbanck, P., Massotte, L. & Dresse, A. (1989) *Eur. J. Pharmacol.* **164,** 373–376.
55. Bartfai, T., Bedecs, K., Land, T., Langel, Ü., Bertorelli, R., Girotti, P., Consolo, S., Xu, X., Wiesenfeld-Hallin, Z., Nilsson, S., *et al*. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 10961–10965.
56. Melander, T., Hökfelt, T., Rökaeus, Å., Cuello, A. C., Oertel, W. H., Verhofstad, A. & Goldstein, M. (1986) *J. Neurosci.* **6,** 3640–3654.
57. Holets, V. R., Hökfelt, T., Rökaeus, A., Terenius, L. & Goldstein, M. (1988) *Neuroscience* **24,** 893–906.
58. Lundberg, J. M. & Hökfelt, T. (1993) *Trends Neurosci.* **6,** 325–333.
59. Grenhoff, J., Nisell, M., Ferré, S., Aston-Jones, G. & Svensson, T. H. (1993) *J. Neural Transm.* **93,** 11–25.
60. Svensson, T. (1999) *Acta Psychiatr. Scand.*, in press.