Cellular Localization of GABA<sub>A</sub> Receptor α Subunit Immunoreactivity in the Rat Hypothalamus: Relationship With Neurones Containing Orexigenic or Anorexigenic Peptides

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Key words: GABA, hypothalamus, food intake, body weight, immunohistochemistry.

Abstract

γ-Aminobutyric acid (GABA), the major inhibitory neurotransmitter in the brain, acts via two different types of GABA receptors. GABA<sub>A</sub> receptors are composed of five subunits that belong to eight different classes. Depending on their subunit composition, distinct pharmacological and electrophysiological properties are obtained. GABA is produced in certain hypothalamic neurones known to be involved in control of feeding behaviour. We report the detailed immunohistochemical localization of four GABA<sub>A</sub>α subunits in hypothalamic regions associated with the regulation of feeding behaviour. Immunoreactive structures for all studied GABA<sub>A</sub>α subunits were observed in the hypothalamus, but with subunit-specific staining patterns. GABA<sub>A</sub>α<sub>1</sub> immunoreactivity was most prominent in the dorsomedial hypothalamic nucleus and in the lateral hypothalamic area (LHA), whereas GABA<sub>A</sub>α<sub>2</sub>, α<sub>3</sub> and α<sub>5</sub> subunits exhibited particularly strong immunoreactivity in the ventromedial hypothalamic nucleus. In comparison, GABA<sub>A</sub>α subunit immunoreactivities were generally weak in the arcuate nucleus. In the ventromedial part of the arcuate nucleus, neuropeptide Y- and agouti-related peptide-containing cell bodies, which also are known to be GABAergic, were immunoreactive for only the GABA<sub>A</sub>α<sub>3</sub> subunit, whereas pro-opiomelanocortin- and cocaine- and amphetamine-regulated transcript- containing cell bodies located in the ventrolateral subdivision of the arcuate nucleus, showed GABA<sub>A</sub>α<sub>1</sub>, α<sub>2</sub> and α<sub>3</sub> subunit immunoreactivity. In the LHA, GABA<sub>A</sub>α<sub>2</sub> subunit immunoreactivity was demonstrated in both melanin-concentrating hormone (MCH) and orexin-containing neurones. In addition, MCH neurones contained GABA<sub>A</sub>α<sub>2</sub> immunoreactivity. In neurones of the tuberomammillary nucleus, GABA<sub>A</sub>α<sub>2</sub> and α<sub>5</sub> subunits were colocalized with histidine decarboxylase, a marker for histamine-containing neurones.

Introduction

The γ-aminobutyric acid (GABA)ergic system is the major contributor of the inhibitory tone throughout the mammalian central nervous system (1). GABA<sub>A</sub> mediates its effects by activating two types of receptors; the GABA<sub>A</sub> receptor (GABA<sub>A</sub>R) and the GABA<sub>B</sub> receptor (GABA<sub>B</sub>R). GABA<sub>A</sub>Rs are chloride ion channels that mediate fast synaptic transmission and belong to a superfamily of pentameric ligand-gated ion channels (2, 3), whereas GABA<sub>B</sub>Rs belong to the family of seven-transmembrane receptors (4, 5).

GABA<sub>A</sub>R represents one of the most complex receptor systems due to the heterologous assembly of receptors from a repertoire of subunits (α<sub>1</sub>–6, β<sub>1</sub>–4, γ<sub>1</sub>–3, δ, ε, π, θ and ρ<sub>1</sub>–3), encoded by at least 20 genes into distinct heteromeric receptor complexes (3, 6). Even assuming that a functioning GABA<sub>A</sub>R requires a combination of at least one α, one β and one γ subunit (7), the number of different subunits renders the possibility of the constitution of a large number of pentameric GABA<sub>A</sub>R combinations. However, experiments using subunit-specific antibodies to immunoprecipitate native receptor molecules, together with experiments expressing combinations of subunit cDNAs in mammalian cells, suggest that a finite number of GABA<sub>A</sub>R subtypes exists in the brain (8, 9). In situ hybridization and immunohistochemical studies show that functionally distinct neurones express different GABA<sub>A</sub>R subunits (10–12). Among the GABA<sub>A</sub>α subunits, the α<sub>1</sub> subunit is most widely distributed (10–12) and is practically present in all brain regions. However, in the hypothalamus, the GABA<sub>A</sub>α<sub>2</sub> subunit is the predominant...
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GABA_A receptors in hypothalamus

To reveal the chemical identity of GABA_A receptor-immunoreactive (ir) neurones, direct double-labelling was performed by combining rabbit polyclonal GABA_A_R α subunit variant (10, 12). The GABA_A_R α, β, and γ subunit mRNAs are also found in hypothalamic nuclei, but in lower amounts (12).

The hypothalamus is a vital centre which serves to maintain homeostasis, including the control of food intake and body weight (13, 14). There is a dense network of GABAergic terminals within the entire hypothalamus, and GABA is synthesized in neurones of several hypothalamic nuclei (1), which are known to be involved in control of feeding behaviour (15). GABA has been described as an orexigenic neurotransmitter (15), acting through both GABA_A and GABA_B receptors (16–21). To increase our understanding of the presence of scattered GABA_A receptors, we studied the detailed cellular localization of GABA_A, GABA_B, and GABA_C receptors (16, 17, 22) using immunohistochemistry. To define the chemical identity of hypothalamic GABA_A-receptor-immunoreactive neurones, we employed a direct double-labelling technique, combined with confocal microscopy, to study colocalization of GABA_A receptors with hypothalamic peptides, which have been implicated as important regulators of food intake.

Materials and methods

Animals and tissue preparations

Males Sprague-Dawley rats (weight 150–200 g; B & K Universal, Stockholm, Sweden) were used. The rats were kept for at least 1 week under a 12 : 12 h light/dark cycle (lights on at 06:00 h) in a temperature-controlled room and had free access to food pellets and tap water. The experiments were approved by the local ethical committee for animal experiments, Stockholm, Sweden. The rats were anaesthetized with sodium pentobarbitone (injected intraperitoneally, 40 mg/kg; Apoteket Produktion & Laboratorier, Umeå, Sweden) and perfused via the ascending aorta with 50 ml of Ca^2+–free Tyrode’s solution (37 °C), followed by 50 ml of formalin-picric acid fixative (37 °C) (4% paraformaldehyde and 0.4% picric acid in 0.16 M phosphate buffer, pH 6.9). Perquisitions were thereafter continued for 6 min with ice-cold fixative. Some rats received an injection of colchicine (120 μg in 20 μl 0.9% NaCl; Sigma, St Louis, MO, USA) into the lateral ventricle 24 h before perfusion. Colchicine arrests axonal transport, thereby increasing levels of transmitters, enzymes and peptides/proteins in the cell soma (22).

The brains were removed and postfixed in the same fixative for 90 min at 4 °C and rinsed for at least 24 h in 0.1 M phosphate buffer (pH 7.4) containing 10% sucrose, 0.02% bacitracin (Sigma) and 0.01% sodium azide (Riedel-de Haen, Seelze, Germany). The brains were frozen and 10 μm coronal sections were cut in a cryostat (Microm HM560, Walldorf, Germany).

Immunohistochemistry

Sections were incubated overnight at 4 °C with rabbit polyclonal antiserum to GABA_A_R α subunit (diluted 1 : 20,000), guinea pig polyclonal antiserum to GABA_A_R γ subunit (diluted 1 : 2,500), GABA_A_R β subunit (diluted 1 : 2,500) or GABA_A_R β subunit (diluted 1 : 2,500). All GABA_A_R subunit specific antisera have been extensively characterized (23–27) and their specific antisera have been extensively characterized (23–27) and their suitability for immunohistochemistry has been documented previously (10, 26–30). The antisera were diluted in 0.3% Triton X-100, 0.01% sodium azide (Riedel-de Haen), 0.02% bacitracin (Sigma) in phosphate-buffered saline (PBS; 0.1 M phosphate buffer; pH 7.4; 0.15 M NaCl). The sections were rinsed in PBS and incubated with Cy3-conjugated donkey anti-rabbit or antiguinea pig secondary antibodies (diluted 1 : 250; Jackson ImmunoResearch, West Grove, PA, USA) for 1 h at room temperature.

Results

General aspects

Incubation with antisera to GABA_A_R subunits α1, α2, γ1 and γ2 revealed immunoreactivity in many areas of the hypothalamus (Figs 1A–I and 2A–I). However, there were several differences in the staining patterns obtained with the GABA_A_R subunit-specific antisera. Within the paraventricular nucleus (PVN), only weak to moderate fluorescence intensity was demonstrated for all the analysed subunits (Fig. 1A–D). There were several GABA_A_R α1, α2, γ1 and γ2-immunoreactive cell bodies in the magnocellular division of the PVN (Fig. 1E–I). In the arcuate nucleus, and in its ventromedial aspect in particular, GABA_A_R α1, α2, γ1 and γ2 immunoreactivity exhibited a weak staining pattern in comparison with other areas at mid-hypothalamic levels (Fig. 2A–D). In the ventromedial hypothalamic nucleus (VMH), immunopositive cell bodies were found for all GABA_A_R α subunits (Fig. 2E–I); however, GABA_A_R α1 immunoreactivity was weaker compared with GABA_A_R subunits α2, γ1 and γ2 (Fig. 2A–D).

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compared to the GABAAR subunits $\alpha_2$, $\alpha_3$ and $\alpha_5$, which displayed a weak staining in these areas (Fig. 2n–d). However, stronger GABAAR $\alpha_2$-ir was also present in ventral parts of the DMH (Fig. 2n). A distinct border was observed between the internal and external layers of the median eminence, where all subtypes showed stronger immunoreactivity in the internal layer (Fig. 2a–d).

There were differences in the subcellular localization of GABAAR immunoreactivity among different types of neurones. At high magnification, the GABAAR $\alpha$ subunit-specific antiserum labelled numerous, very fine elements throughout the neuropil, but also individual soma and their dendrites. There were patches of immunoreactive material in individual GABAAR $\alpha$ subunit-positive neurones, presumably corresponding to receptor aggregates. In most GABAAR $\alpha$ subunit-ir neurones, the immunolabelled aggregates increased in numbers to such an extent that the membrane appeared to be continuously labelled; however, some aggregates were of higher intensity and size. Within the arcuate nucleus, staining of the GABAAR $\alpha_1$, $\alpha_2$ and $\alpha_3$ subunits was predominantly observed in the periphery of individual cell bodies, presumably representing an association with the plasma membrane (Figs 3a,c,e, 4a,c, 5a,c,e and 6a,c,e), whereas the GABAAR $\alpha_5$ subunit immunoreactivity in addition showed a cytoplasmatic punctate staining (Figs 3g, 4c, 5g and 6g). The amount of cytoplasmatic staining obtained with the antisera to the different GABAAR subunits varied greatly among different types of neurones and $\alpha$ subunits. Within the LHA, GABAAR $\alpha_1$ and $\alpha_2$ subunits were primarily localized to the plasma membrane, whereas both GABAAR $\alpha_3$ and $\alpha_5$ subunit immunoreactivities showed a cytoplasmatic staining (Figs 7a–f and 8a–f). GABAAR $\alpha_3$ subunit immunoreactivity was also detected primarily in the periphery of individual neurones in the tuberomammillary nucleus (TMN) (Fig. 9a), whereas the GABAAR $\alpha_5$ subunit was distributed in the cytoplasm (Fig. 9e). GABAAR $\alpha_1$- and GABAAR $\alpha_3$-positive neurones were not detected in the TMN.

In untreated rats, direct double-labelling showed that GAD65-ir nerve fibres and terminals surrounded and were present in close association with GABAAR $\alpha_2$, $\alpha_3$ and $\alpha_5$ subunit-ir cell bodies located in the arcuate nucleus as well as in the LHA (data not shown).

Chemical identity of GABAAR-ir neurones

For the studies in which the GABAAR $\alpha$-immunoreactive neurones were chemically defined, an approximate number of 15–10 sections were examined for each combination.

In colchicine-treated rats, NPY- and AGRP-containing cell bodies located in the ventromedial arcuate nucleus were demonstrated to contain GABAAR $\alpha_3$ immunoreactivity (Figs 3e,f and 4c,d), but lacked GABAAR $\alpha_1$, $\alpha_2$ and $\alpha_5$ immunoreactivity (Figs 3a–d, 4g,h and 4a,b,e,f). By contrast, most of the larger POMC- and CART-containing neurones located in the ventrolateral part of the arcuate nucleus were shown to contain many of the investigated GABAAR $\alpha$ subunits (Figs 5a–h and 6a–h). Thus, GABAAR $\alpha_1$, $\alpha_2$ and $\alpha_5$ subtypes were all present in the periphery of individual POMC/CART-positive neurones (Figs 5a–f and 6a–f), whereas GABAAR $\alpha_5$ immunoreactivity was not detected in these neurones (Figs 5g,h and 6g,h). GABAAR $\alpha$-ir neurones located in the arcuate nucleus exhibited immunoreactivity for all studied GABAAR $\alpha$ subtypes (data not shown). Many large MCH- or orexin-ir neurones in the LHA contained GABAAR $\alpha$ subunit immunoreactivity. Thus, GABAAR $\alpha_2$ and $\alpha_5$, but not GABAAR $\alpha_3$ subtype immunoreactivity was present in most MCH-ir neurones in the LHA (Figs 7a,b,c–f). Orexin-containing cell bodies were GABAAR $\alpha_3$-ir (Fig. 8c–d), but not GABAAR $\alpha_2$- or $\alpha_5$-ir (Figs 8a,b,e,f). The few GAD-positive cell bodies seen in the LHA were not GABAAR $\alpha_1$- or $\alpha_2$-ir (data not shown). However, GAD immunoreactivity was demonstrated in a few GABAAR $\alpha_2$-ir cell bodies in this region (data not shown). The majority of the tuberomammillary histaminergic cell bodies, identified with an antiserum to HDC, were shown to contain GABAAR $\alpha_2$ and $\alpha_5$ immunoreactivity (Figs 9a,b,e,f). GABAAR $\alpha_5$ immunoreactivity was weak and presumably located in nerve fibres projecting to tuberomammillary neurones and not localized to the cell soma (Figs 9c,d).

Discussion

General aspects

The present results show a widespread distribution of GABAAR $\alpha$ subunit immunoreactivity in the rat hypothalamus in agreement with previously published data (10). It has been reported that GABAAR $\alpha_4$ and $\alpha_6$ subunit mRNAs are not expressed in the hypothalamus, these subunits were not included in this study (12). The presence of different GABAAR $\alpha_1$, $\alpha_2$, $\alpha_3$ and $\alpha_5$ subunits in neurones known to be involved in ingestive behaviour suggests a GABAergic influence via GABAARs on hypothalamic neuronal pathways regulating feeding behaviour (see below).

Cellular localization of GABAAR $\alpha$ subunit immunoreactivity

The GABAAR $\alpha_1$ subunit has been described as the most abundant $\alpha$ subunit in adult brain (23, 33–35). However, in
Fig. 3. (A–H) Images obtained via confocal microscopy of sections of the rat arcuate nucleus after direct double-labelling combining rabbit antiserum to GABA_AR α_1 (A) with mouse monoclonal antibodies to neuropeptide Y (NPY) (B) and guinea-pig antiserum to GABA_AR α_2, α_3 and α_5 (C,E,G) with rabbit antiserum to NPY (D,F,H). All studied subtypes are expressed in the ventromedial part of the arcuate nucleus (A,C,E,G). Comparison of (A,C,E,G) with (B,D,F,H), respectively, shows that there are GABA_AR α_3-ir neurones that contain NPY (thick arrows). There are also GABA_AR α_1, α_2, α_5 and α_5-positive neurones that are NPY-negative (thin arrows) or GABA_AR α_1, α_2, α_3 and α_3-negative neurones that are NPY-positive (short arrows). Scale bars = 5 μm.
Fig. 4. (A–F) Images obtained via confocal microscopy of sections of the rat arcuate nucleus after direct double-labelling combining guinea-pig antiserum to GABA\textsubscript{A}R \(\alpha_2\), \(\alpha_3\) and \(\alpha_5\) (A–C,E) with rabbit polyclonal antiserum to agouti-related peptide (AGRP) (B–D,F). All studied subtypes are expressed in the ventromedial part of the arcuate nucleus (A–C,E). Comparison of (A–C,E) with (B–D,F), respectively, shows that there are GABA\textsubscript{A}R \(\alpha_3\)-ir neurones that contain AGRP (thick arrows). There are also GABA\textsubscript{A}R \(\alpha_2\), \(\alpha_3\) and \(\alpha_5\)-positive neurones that are neuropeptide Y (NPY)-negative (thin arrows) or GABA\textsubscript{A}R \(\alpha_1\), \(\alpha_2\), \(\alpha_3\) and \(\alpha_5\)-negative neurones that are NPY-positive (short arrows). Scale bars = 5 \(\mu\)m.
the hypothalamus, the GABA$_\alpha$R $\alpha_2$ subunit appears to be the most dominating $\alpha$ subunit (10, 12). It has been described that the GABA$_\alpha$R $\alpha_1$ and GABA$_\alpha$R $\alpha_2$ subunits display an approximately complementary distributions; GABA$_\alpha$R $\alpha_1$ subunit immunoreactivity is prominent in regions where GABA$_\alpha$R $\alpha_2$ subunit immunoreactivity is absent or weak (10). This observation is in agreement with the present findings. In accordance with a previous study by Fritschy and Möhler (10), the PVN exhibited only weak to moderate staining for all the subunits analysed. In this study, we detected several GABA$_\alpha$R $\alpha_2$, $\alpha_3$ and $\alpha_5$ subunit-ir cell bodies in the magnocellular part of the PVN.

The GABA$_\alpha$R $\alpha_1$ subunit antisera exhibited the strongest immunoreactivity of the studied subunits and the staining pattern was different as compared with GABA$_\alpha$R $\alpha_2$, $\alpha_3$ and $\alpha_5$ subunits at the mid-hypothalamic level. GABA$_\alpha$R $\alpha_1$ subunit exhibited strong immunoreactivity in the DMH and in the LHA, whereas GABA$_\alpha$R $\alpha_2$, $\alpha_3$ and $\alpha_5$ subunits displayed strong immunoreactivity in the VMH. It is important to note that there was only weak fluorescence intensity for all studied GABA$_\alpha$R $\alpha$ subunits in the arcuate nucleus, although the immunoreactivity appeared to be stronger in the ventrolateral subdivision of the nucleus compared to the ventromedial part.

The subcellular localization of the GABA$_\alpha$R $\alpha$ subunits studied varied between different GABA$_\alpha$R $\alpha$ subunit and hypothalamic regions. These differences may suggest possible variations in receptor turnover and or reflect presence of a pool of receptor proteins not being inserted into the plasma membrane. In addition, it can not be excluded that colchicine treatment effects the cellular staining of GABA$_\alpha$R $\alpha_1$, $\alpha_2$, $\alpha_3$ and $\alpha_5$ subunits because it has been shown that exposure of cultured neurones to colchicine appears to produce a stronger cytoplasmatic immunostaining of the GABA$_\alpha$R $\alpha$ subunits without affecting the total cellular level of the proteins (36). However, we did not detect any obvious differences in cellular staining for the different GABA$_\alpha$R $\alpha$ subunits when comparing untreated and colchicine-treated rats.

In accordance with earlier studies, we detected a regional codistribution of GABA$_\alpha$R $\alpha$ subunits in rat hypothalamic neurones (10, 11). In spite of the fact that different GABA$_\alpha$R $\alpha$ subunits have been demonstrated to be colocalized in neurones with histochemical methods (10), it cannot be concluded whether the neuron-specific colocalization demonstrates that the GABA$_\alpha$R $\alpha$ subunits are part of the same receptor or, alternatively, are in different GABA$_\alpha$R complexes.

Chemical identity of GABA$_\alpha$R $\alpha$ subunit-ir cell bodies

One or more of the studied GABA$_\alpha$R $\alpha$ subunit immunoreactivities were observed in neurones containing mediators that stimulates or inhibits food intake. GABA$_\alpha$R $\alpha_1$, $\alpha_2$, $\alpha_3$ and $\alpha_5$ subunit immunoreactivities were found in the cell bodies of the arcuate nucleus. However, there were obvious differences among two subpopulations of neurones within the nucleus. NPY/AGRP-containing neurones were only positive for the GABA$_\alpha$R $\alpha_1$ subunit and thus lacked GABA$_\alpha$R $\alpha_2$, $\alpha_3$ and $\alpha_5$ expression. On the other hand, POMC/CART-containing neurones were positive for GABA$_\alpha$R $\alpha_1$, $\alpha_2$ and $\alpha_3$ subunit immunoreactivity. These results suggest that the ventromedial POMC/CART neurones may be the main targets for GABA, whereas the NPY/GABA neurones are not. In this context, it is important to point out that there is a clear difference between the two neuronal population in the arcuate nucleus with regard to the GABAergic expression pattern. NPY/AGRP neurones are GABAergic, whereas POMC/CART neurones appear not to be GABAergic.

In the LHA, there were GABA$_\alpha$R $\alpha_1$-, $\alpha_2$-, $\alpha_3$- and $\alpha_5$-ir neurones. This region contains two food-stimulatory peptides, MCH and orexin, which are synthesized in separate cell populations (37, 38). MCH was colocalized with GABA$_\alpha$R $\alpha_2$ and $\alpha_3$ subunits, whereas orexin-containing neurones only had GABA$_\alpha$R $\alpha_2$ subunit immuno reactivity. Whether or not GABA$_\alpha$R $\alpha_1$ subunit immunoreactivity is present in these two cell populations could not be established because the antiserum to the GABA$_\alpha$R $\alpha_1$ subunit, MCH and orexin, are all raised in rabbits. Consequently, double-labeling could not be performed. In colchicine-treated rats, a few GAD65-positive neurones could be detected in the lateral hypothalamic. We observed colocalization of GAD65 and GABA$_\alpha$R $\alpha_2$ subunit, but not with GABA$_\alpha$R $\alpha_5$ subunits. Furthermore, there were some GAD65-positive neurones that were GABA$_\alpha$R $\alpha_2$-negative. Presumably they may be GABA$_\alpha$R $\alpha_1$-positive because GABA$_\alpha$R $\alpha_1$ antiserum displayed strong immunoreactivity in the LHA. Unfortunately, we were not able to confirm this assumption for the same reasons as described above.

The magnocellular HDC-containing neurones of the TMN were the only cells investigated that contained GABA$_\alpha$R $\alpha_3$ subunit immunoreactivity, although the subunit was seen in all studied hypothalamic areas. In addition, GABA$_\alpha$R $\alpha_2$ subunit was detected in these neurones. However, histamine-containing cell bodies of the TMN did not exhibit GABA$_\alpha$R $\alpha_1$ or $\alpha_3$ subunit immunoreactivity, in accordance with earlier studies (10, 12).

Functional considerations in relation to body weight regulation

GABA-synthesizing neurones located in the ventromedial subdivision of the arcuate nucleus coexpress the orexigenic peptides, NPY and AGRP (39, 40). GABA is also colocalized with NPY and some AGRP-ir nerve terminals within the PVN (41, 42). In agreement, microinjection of muscimol, a GABA$_\alpha$R agonist, into PVN stimulates feeding (17, 18, 41).

Fig. 5. (A–H) Images obtained via confocal microscopy of sections of the rat arcuate nucleus after direct double-labeling combining rabbit antiserum to GABA$_\alpha$R $\alpha_3$ (A) or guinea-pig antiserum to GABA$_\alpha$R $\alpha_2$, $\alpha_3$ and $\alpha_5$ (C,E,G) with mouse monoclonal antibodies to adrenocorticotropic hormone; a marker for pro-opiomelanocortin-(POMC)-containing neurones (B,D,F,H). All studied subtypes are expressed in the ventrolateral part of the arcuate nucleus (A,C,E,G). Comparison of (A,C,E,G) with (B,D,F,H), respectively, shows that there are GABA$_\alpha$R $\alpha_1$-, $\alpha_2$-, $\alpha_3$-ir neurones that contain POMC (thick arrows). GABA$_\alpha$R $\alpha_1$-expressing neurones appears to be POMC-negative (G,H; thin arrows). Some POMC-positive cell bodies are GABA$_\alpha$R $\alpha_2$-, $\alpha_3$-negative neurones [compare short arrows in (c) with (a) and (e) with (f)], whereas all POMC-containing neurones appear to be GABA$_\alpha$R $\alpha_1$-positive (A,a). Scale bars = 5 μm.
Fig. 6. (A–H) Images obtained via confocal microscopy of sections of the rat arcuate nucleus after direct double-labelling combining rabbit antiserum to GABA_A_R α_1 (A) or guinea-pig antiserum to GABA_A_R α_2, α_3 and α_5 (C,E,G) with chicken polyclonal antiserum to cocaine and amphetamine-regulated transcript (CART) (B,D,F,H). All studied subtypes are expressed in the ventrolateral part of the arcuate nucleus (A,C,E,G). Comparison of (A,C,E,G) with (B,D,F,H), respectively, shows that there are GABA_A_R α_1-, α_2-, α_3-ir neurones that contain pro-opiomelanocortin (POMC) (thick arrows). GABA_A_R α_5-expressing neurones appear to be CART-negative (G,H; thin arrows). Some CART-positive cell bodies are GABA_A_R α_2-, α_3-negative neurones [compare short arrows in (C) with (D) and (E) with (F)]. whereas all POMC-containing neurones appear to be GABA_A_R α_1-positive (A,B). Scale bars = 5 μm.
Fig. 7. (A–F) Images obtained via confocal microscopy of sections of the lateral hypothalamic area (LHA) after direct double-labelling combining guinea-pig antiserum to GABA$_\text{A}$R $\alpha_2$, $\alpha_3$, and $\alpha_5$ (A,C,E) with rabbit polyclonal antiserum to melanin-concentrating hormone (MCH) (B,D,F). All studied subtypes are expressed in the LHA (A,C,E). Comparison of (A,C,E) with (B,D,F), respectively, shows that there are GABA$_\text{A}$R $\alpha_2$- and $\alpha_3$-ir neurones that contain MCH (thick arrows), whereas MCH-containing neurones lack GABA$_\text{A}$R $\alpha_5$ [compare short arrows in (E) with (F)]. There are also GABA$_\text{A}$R $\alpha_5$-positive neurones that are MCH-negative (thin arrows). Scale bars = 5 μm.
Fig. 8. (A–D) Images obtained via confocal microscopy of sections of the lateral hypothalamic area (LHA) after direct double-labelling combining guinea-pig antiserum to \( \text{GABA}_{A} \) \( \alpha_{2} \), \( \alpha_{3} \) and \( \alpha_{5} \) (A,C,E) with rabbit polyclonal antiserum to orexin (B,D,F). All studied subtypes are expressed in the LHA (A,C,E). Comparison of (A,C,E) with (B,D,F), respectively, shows that there are \( \text{GABA}_{A} \) \( \alpha_{3} \)-ir neurones that contain orexin (thick arrows), whereas orexin-containing neurones lack \( \text{GABA}_{A} \) \( \alpha_{2} \)- and \( \alpha_{5} \)-immunoreactivity [compare short arrows in (A) with (B) and (E) with (F)]. There are also \( \text{GABA}_{A} \) \( \alpha_{5} \)-positive neurones that are orexin-negative (C,D; thin arrows). Scale bars = 5 \( \mu \)m.
Fig. 9. (A–F) Images obtained via confocal microscopy of sections of the tuberomammillary nucleus (TMN) after direct double-labelling combining guinea-pig antiserum to GABA\(_A\)R \(\alpha_2\), \(\alpha_3\) and \(\alpha_5\) (A,C,E) with rabbit antiserum to histidine decarboxylase (HDC); a marker for histamine-containing neurones (B,D,F). All studied subtypes are expressed in the TMN (A,C,E) (GABA\(_A\)R \(\alpha_1\) not shown). Note that most GABA\(_A\)R \(\alpha_2\)- and \(\alpha_5\)-ir neurones exhibit HDC immunoreactivity [compare thick arrows in (A) with (B) and (E) with (F)]. GABA\(_A\)R \(\alpha_3\)-containing nerve terminals appear to be localized in close contact to HDC-positive neurones (C,D). Scale bars = 5 \(\mu\)m.
Furthermore, this hypothalamic site also gives an orexigenic response to NPY microinjections (43). Because coadministration of NPY and muscimol into the PVN enhanced the feeding over that evoked by NPY or muscimol alone (41), it is likely that GABA\textsubscript{A}R\textsubscript{a} and NPY nerve endings are connecting to the same target cells in the PVN. Whereas neurones located in the ventromedial subdivision of the arcuate nucleus contain orexigenic mediators (44–47), the ventrolaterally located arcuate neurones contain the anorexigenic peptides orexigenic mediators (44–47), the ventrolaterally located arcuate neurones contain the anorexigenic peptides

\textit{GABAAR} combines with at least one GABA\textsubscript{A}R in neurones of the VMH, which is a region suggested to act as a satiety centre (54). Infusion of GABA\textsubscript{A}R agonist into the VMH increases food intake dose-dependently in lean rats and the effect is blocked by local pretreatment with the GABA\textsubscript{A}R antagonist picrotoxin (17). These results suggest that GABA\textsubscript{A}Rs located in the VMH are involved in the feeding system, where activation of GABA\textsubscript{A}Rs inhibits satiety-related neurones. Such neurones may be glutamatergic because the VMH contains many neurones expressing the vesicular glutamate transporter 2 (55). It has also been suggested that GABA influences the development and organization of the VMH (56). GABA\textsubscript{A}R\textsubscript{a} subunit is localized on certain TMN neurones and terminals in the rat CNS as revealed by GAD immunocytochemistry. In: Bjo¨rklund A, Ho¨kfelt T, eds. Handbook of Chemical Neuroanatomy, vol. 4. GABA and Neuropeptides in the CNS, Part 1. Amsterdam: Elsevier, 1985: 436–608.

Acknowledgements

This research was supported by support by EC FP6 funding (contract LSHM-CT-2003-503041), the Swedish Research Council (72X-10358-10A), the National Network in Neuroscience (NNN), Ahlén-stiftelsen, Dr P. Håkanssons stiftelse (Duvani), Knut and Alice Wallenberg Foundation (confocal system), stiftelsen Elsa and Sigurd Goljes Minne, the Swedish Society for Medical Research and funds from Karolinska Institutet. We wish to express our sincere gratitude to Professor Tomas Hökfelt for providing us with antisera for double-labelling.

Accepted 4 May 2004

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