Published in final edited form as:
Clin Chem Lab Med. 2007 ; 45(12): 1777–1784. doi:10.1515/CCLM.2007.342.

Differential expression of γ-aminobutyric acid receptor A ($GABA_A$) and effects of homocysteine

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Abstract

Background—γ-Aminobutyric acid (GABA) is a known inhibitory neurotransmitter in the mammalian central nervous system, and homocysteine (Hcy) behaves as an antagonist for $GABA_A$ receptor. Although the properties and functions of $GABA_A$ receptors are well studied in mouse neural tissue, its presence and significance in non-neural tissue remains obscure. The aim of the present study was to examine the expression of $GABA_A$ receptor and its subunits in non-neural tissue.

Methods—The mice were analyzed. The presence of $GABA_A$ receptor and its subunits was evaluated using Western blot and reverse transcription polymerase chain reaction.

Results—We report that $GABA_A$ receptor protein is abundant in the renal medulla, cortex, heart, left ventricle, aorta and pancreas. Low levels of $GABA_A$ receptor protein were detected in the atria of the heart, right ventricle, lung and stomach. The mRNA protein expression of $GABA_A$ receptor subunit shows that α1, β1, β3 and γ1 subunits are present only in brain. The mRNA protein expression levels of $GABA_A$ receptor α2, α6, β2 and γ3 subunits were highly expressed in brain compared to other tested tissue, while $GABA_A$ receptor γ2 subunit was expressed only in brain and kidney. Treatment of microvascular endothelial cells with Hcy decreased $GABA_A$ receptor protein level, which was restored to its baseline level in the presence of $GABA_A$ receptor agonist, muscimol. The distribution of $GABA_A$ and $GABA_B$ receptors in wild type mice was determined and tissue-specific expression patterns were found showing that several receptor subtypes were also expressed in the central nervous system.

Conclusions—Hcy, a $GABA_A$ agonist, was found to decrease $GABA_A$ expression levels. These data enlarge knowledge on distribution of GABA receptors and give novel ideas of the effects of Hcy on different organs.

Keywords

brain; γ-aminobutyric acid receptor A ($GABA_A$); heart; homocysteine; kidney; lung; mouse; muscimol; pancreas

Introduction

Homocysteine (Hcy) is a thiol-containing amino acid derived from the metabolic demethylation of dietary methionine. Elevated levels of Hcy, hyperhomocysteinemia

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(HHcy) is associated with vascular dementia and Alzheimer's diseases. In animals, HHcy causes seizures; moreover, Hcy transduces intracellular nuclear receptors (1). It binds to γ-aminobutyric acid (GABA) receptor, and therefore competes with muscimol (agonist of $GABA_A$ receptor) and baclofen (agonist of $GABA_B$ receptor) (2, 3).

GABA, an endogenous amino acid, is present in vertebrate peripheral (4–6) and central nervous system (7, 8). This amino acid is a major functionally inhibitory neurotransmitter in the nervous system (9). It also mediates various functional responses in non-neuronal tissue (8, 10–13). The formation of GABA occurs by the decarboxylation of glutamate, catalyzed by glutamate decarboxylase (GAD) enzyme. Biochemical studies have demonstrated the significant presence of GAD enzyme in the nervous system, as well as in endocrine organs (8, 10).

GABA receptors are divided into two distinct groups: $GABA_A$ and $GABA_B$ (14). These receptors differ by their affinity for specific agonist and antagonist profiles and separate modes of action (9, 15). $GABA_A$ receptor is a ligand-gated chloride channel that exists in a hetero-pentameric structure from a number of subunits (16, 17). In rodents, $GABA_A$ receptors comprise seven different classes of subunits: α1–α6, β1–β3, γ1–γ3, ρ1–ρ3, ε, θ, δ (18–21). $GABA_A$ receptors are composed of α, β and γ subunits (22). Another receptor, $GABA_B$, is a G-protein coupled receptor. The activation of $GABA_B$ causes an inhibition of adenylate cyclase activity, a decrease in calcium and an increase in K^+ conductance in neuronal membranes (23–25). $GABA_B$ receptor is activated by baclofen (2), while its inhibition is mediated by indirect gating of either potassium or chloride channels (25). $GABA_A$ receptors play a significant role in general anesthesia, feeding, cardiovascular regulation, anxiolytic effects and anticonvulsive activity (3). However, the existence of $GABA_A$ receptor outside the central nervous system is not well studied. Activation of $GABA_A$ receptors protect against many types of seizures and convulsions in animals and humans. To date, this receptor is a target for a number of drugs with significant clinical importance.

Recent studies on rats showed that the receptor-channel complex may exist outside the central nervous system (26, 27). These studies have indicated that agonists of the GABA receptor bind to various tissues outside the central nervous system, including heart, kidney and pancreas (19, 28, 29). However, no $GABA_A$ receptors were identified in mouse non-neural tissue. As mouse is a commonly used experimental animal, and $GABA_A$ receptor may be involved in many functional aspects of a microcirculation and vascular remodeling, the objective of the present study was to identify and characterize $GABA_A$ receptor in various organs of mouse.

Materials and methods

Materials

DL-Hcy, muscimol, β-mercaptoethanol, proteinase inhibitors [phenylmethylsulfonyl fluoride (PMSF), aprotinin, leupeptin, and pepstatin], tribromoethanol, fibronectin, agarose, collagenase type B (0.2%), antibodies against $GABA_A$ receptor and β-actin were purchased from Sigma (St. Louis, MO, USA), specific antibodies against $GABA_A$ receptor subunits were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Dulbecco's modified Eagle's medium (DMEM) was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA), while DMEM/Ham's F-12 50:50 mix (DMEM/F12-50/50) was purchased from Cell Grow Media-tech, Inc. (Herndon, VA, USA). Fetal bovine serum was purchased from HyClone (Logan, UT, USA). Trypsin (0.25%)/EDTA, penicillin, streptomycin, L-glutamate, heparin and trizol were obtained from

Invitrogen-Gibco (Carlsbad, CA, USA). Complete MCDB131 medium was purchased from VEC Technology, Inc. (Rensselaer, NY, USA). Bio-Rad protein assay reagents were purchased from Bio-Rad (Hercules, CA, USA).

Animals

Wild type C57BL/6J, 10–12-week-old male mice were purchased from Jackson Laboratories (Bar Harbor, MN, USA). Animal protocols were performed in accordance with the National Institutes of Health guidelines and the IACUC protocol specifications at the University of Louisville for animal safety. All of the animals were fed rodent chow diet and kept in cages in a temperature- and humidity-controlled room with a 12-h light/dark cycle.

Tissue collection

Mice were anesthetized with 0.02 mL/g body weight tribromoethanol (intraperitoneally). The brain, heart, lung, kidney, aorta, stomach and pancreas were excised, and tissues were rapidly washed twice in phosphate buffered saline (PBS). The tissue samples were stored at −20°C until further use.

Preparation of samples

The tissue samples were homogenized in 300 μL of ice-cooled lysis buffer (pH 7.4) containing Tris-HCl (20 mM), EDTA (1 mM), NaCl (50 mM), dithiothreitol (1 mM), β-mercaptoethanol (10 mM) and proteinase inhibitors pepstatin (100 μM), aprotinin (10 μM), leupeptin (100 μg/mL) and PMSF (1 mM), which were added prior to use. Cells were lysed in ice-cold-modified RIPA lysis buffer (pH 7.4) containing: Tris-HCl (50 mM), NP-40 (1%), Na-deoxycholate (0.25%), NaCl (150 mM), EDTA (1 mM), PMSF (1 mM) aprotinin, leupeptin, pepstatin (1 μg/mL each), Na_3VO_4 (1 mM) and NaF (1 mM). The clear supernatant of tissue and cell samples was collected, and protein concentration in the samples was determined by Bradford's method (29) using bovine serum albumin as a standard.

Endothelial cell culture

Mouse brain microvascular endothelial cells (MBMEC) were purchased from ATCC. Cells were grown in modified DMEM (ATCC) with 10% fetal bovine serum. Mouse aortic endothelial cells (MAEC) were a generous gift from Dr. Kathleen Bove (Stratton VA Medical Center, Albany, NY, USA). The cells were maintained in complete DMEM/F12-50/50 mix and supplemented with 10% fetal bovine serum (30). Mouse cardiac endothelial cells (MCMEC), which were isolated as described previously (31), were grown in fibronectin-coated wells in complete MCDB131 medium with 10% fetal bovine serum.

Mouse lung endothelial (MLMEC) were isolated from lungs of wild type mice using a modification of a method described earlier (31). Briefly, both lungs from two mice were removed aseptically, rinsed in Hank's balanced salt solution (HBSS; Invitrogen-Gibco) to remove excess blood, and minced into ~2 mm square pieces. The lung pieces were digested in 10 mL of collagenase type B for 25 min at 37°C with occasional agitation. Further digestion was carried out with 1 mL of 0.25% trypsin/EDTA for 5–10 min at 37°C. The cellular digest was filtered through sterile 40-μm nylon mesh and then centrifuged at 120×*g* for 5 min. After the supernatant was removed, the cell pellet was washed twice in DMEM containing 20% fetal bovine serum. MLMEC were resuspended in growth media and were grown in humidified conditions at 5% CO_2 at 37°C, as described previously (32). All cells were grown at 37°C with 5% CO_2, 95% air until they became 70%–80% confluence. All experiments were performed between passages 5 and 8.

Western blot analysis

Expression of $GABA_A$ receptor and its subunits was determined by Western blot analysis. Protein samples were mixed with 1:1 v/v ratio with 2× sample loading buffer, boiled at 100°C for 5 min, cooled and then centrifuged at 10,000×*g* for 5 min. Equal amounts of protein (50 μg) for each group were resolved by 4%–15% gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were then electrophoretically transferred to a polyvinylidene fluoride membrane (BioRad, Hercules, CA, USA). Transferred proteins were blocked with 5% non-fat dry milk in TBS-T (pH 7.4) containing Tris-HCl (50 mM), NaCl (150 mM), Tween-20 (0.1%) for 1 h at room temperature. The blots were then incubated with respective primary antibodies in blocking solution, according to the manufacturer's recommendations. After incubation, the blots were washed with TBS-T three times for 10 min each. The blots were incubated with appropriate horseradish peroxidase-conjugated secondary antibody for 1 h. After washing four times (each wash for 10 min) ECL Plus substrate (Amersham Biosciences, Pittsburgh, PA, USA) was applied to the blots for 5 min. To confirm an equal loading of the samples, each blot was probed with mouse anti-rabbit antibody directed against β-actin. The protein bands were visualized by exposing the blots to an X-ray film, as described previously (32). Image analysis of the protein bands was performed using UMAX PowerLook II (Tawin, Republic of China). The protein expression intensity was assessed by integrated optical density (IOD). To account for possible differences in total protein load, the results of the measurements were presented as a ratio of IOD of $GABA_A$ receptor protein in a tissue to the IOD of the respective β-actin.

RNA preparation and semi-quantitative reverse transcription polymerase chain reaction (RT-PCR)

Expression of $GABA_A$ receptor subunits was carried out by assessing an expression of their respective mRNAs. The extraction of total RNA from brain, heart, kidney and lung was isolated, frozen and pulverized under liquid nitrogen, followed by homogenization in trizol reagent (Invitrogen-Gibco), as described earlier (33). Briefly, the concentration of total RNA was quantified by measuring the absorbance at 260 nm. Samples with a peak area ratio of 28S-to-18S rRNA >2.0 were used. RNA (2 μg) was reverse transcribed (RT) using oligo dT primers with a total reaction volume of 20 μL. The RT program was 25°C for 10 min, 42°C for 50 min, and then 70°C for 15 min. PCR was performed using 2 μL of each RT product (cDNA), with a total reaction volume of 20 μL. The PCR thermal cycle was carried out at 94°C for 2 min, and then 35 cycles were carried out at 94°C for 30 s, at 55°C for 30 s, and finally at 72°C for 60 s. This was followed by a final extension for 5 min at 72°C. Oligonucleotide primers specified in Table 1 were synthesized, according to the published sequences (34). Each sample (20 μL) of PCR product together with negative controls were subjected to electrophoresis on 1% agarose gel and stained with ethidium bromide.

Statistical analysis

Data were expressed as mean±SEM, n=4 animals from each group. The arbitrary densitometry unit was represented as percentage relative to control. The data were analyzed by Student's t-test for comparison of the results between the Hcy-treated and non-treated groups. A p-value −0.05 was considered statistically significant.

Results

The results of the present study show that expression of $GABA_A$ receptor was greater in mouse brain than in other tested tissue, such as pancreas, lung and stomach (Figure 1). In kidney, $GABA_A$ receptor was found to be expressed more in the medulla as compared to the cortex region of the kidney (Figure 2). $GABA_A$ receptor protein was present in the whole

heart, with greater expression in the left ventricle and aorta and with a less expression in the atria and right ventricle (Figure 3).

In brain, $GABA_A$-α2 and -α6 subunit mRNAs expression levels were significantly greater than α1 subunit mRNA (Figure 4A, B). $GABA_A$-α1 subunit mRNA was only expressed in brain and was not found in any other tested tissues. The mRNA expression level of α2 subunit was similar in heart, lung and whole kidney. The mRNA expression level of α6 subunit was greater in whole kidney as compared to heart or lung (Figure 4A, B).

The mRNA $GABA_A$ receptor β1 and β3 subunit mRNAs were only expressed in brain and were not found in other tested tissue (Figure 5A, B). Although β2 subunit mRNA of a $GABA_A$ receptor was found in brain, lung, heart and whole kidney, its expression was greater in brain and lung compared to that in other tested tissue (Figure 5A, B).

The γ1 subunit mRNA was not detectable in heart, lung or whole kidney (Figure 6A). It was expressed only in brain (Figure 6A). However, the γ2 subunit mRNA was expressed in all the tested tissues except heart (Figure 6B). We found an ample expression of γ3 subunit mRNA in brain and lung compared to that in heart or kidney (Figure 6B). An ubiquitous gene GAPDH was present in all the tested tissues.

The functional studies on ECs show that Hcy diminishes and muscimol enhances $GABA_A$ receptor expression in all the EC lines: brain (Figure 7A, B), aorta (Figure 7C, D), cardiac (Figure 8A, B) and lung (Figure 8C, D). Although, muscimol did not apparently restore Hcy abrogated expression of $GABA_A$ receptor in MLMEC cell line (Figure 8C, D). This may explain the differences in lung vs. cardiac ECs.

Discussion

Recent studies have indicated that muscimol, a specific agonist of the $GABA_A$ receptor, binds to various tissues outside the central nervous system. This includes renal system and endocrine organs, such as ovaries, adrenal glands and pancreas in rat or guinea pig (5, 8, 10, 13, 35–37). These data suggest that the GABA receptor and its subunits may exist in non-neural tissues (21). However, as of today no direct evidence is available showing that $GABA_A$ receptors are expressed in any other tissue of mouse other than the brain. The results of the present study coincide with studies of others (19, 20, 38) showing that $GABA_A$ receptor and its subunits α1, α2, α6, β1, β2, β3, γ1, γ2 and γ3 are expressed in the mouse brain. However, we found that α1, α2, α6, β1, β2, β3, γ1, γ2 and γ3 subunits of the $GABA_A$ receptor are also present in various non-neural tissues. These $GABA_A$ receptor subunits were most abundant in the mouse brain and kidney.

Sarang et al. showed that mRNA of $GABA_A$ receptor β2 and β3 subunits is expressed only in rat, rabbit and human kidney (21). However, their studies did not explore the protein and mRNA expression of other subunits in other non-neural tissue of these species, as well as in mice. The $GABA_A$ receptor β3 subunit was found primarily in the cortex and medulla of rat (21), and the β2, β3 subunits are transiently expressed in the rat proximal tubule (38, 39). Auto-radiography with use of the $GABA_A$ receptor agonist muscimol demonstrated specific binding to convoluted proximal tubules of the rat renal cortex (40). However, α1 subunit of the $GABA_A$ receptor was identified in the thick ascending limb of the loop of Henle, but not in the proximal tubule or in glomeruli in rats (41). In the present study, we found that $GABA_A$ receptor and its subunits (α2, α6, β2, γ2, γ3) are present in mouse renal system and other non-neural tissues, such as lung and heart. Although β2 and γ2 subunits were expressed in kidney, their expression was not as significant as α6, β2 and γ3 subunits. Interestingly, $GABA_A$ receptor protein expression was greater in medulla as compared to renal cortex (Figure 2).

Recently, $GABA_A$ receptor ε and π subunits were identified in heart and uterus of guinea pig (19, 20). Autoradiographic studies on the (←←3H) GABA binding sites showed presence of $GABA_A$ receptor in rat atria (39, 42). It has been suggested that $GABA_A$ receptors are to be found on the blood vessel membranes (36).

The present study showed for the first time that $GABA_A$ receptor is expressed in the adult mouse heart. It is present in the atria, left ventricle and descending aorta. The less expression of $GABA_A$ receptor was found in the right ventricle. $GABA_A$ receptor α2, α6, β2 and γ3 subunits were also present in adult mouse heart, whereas α1, β1, β3, γ1 and γ2 were not found in the heart.

Tanaka identified $GABA_A$ receptor in the lungs of guinea pig (8). Our data show that some $GABA_A$ protein was visible in mouse lung. Moreover, we found $GABA_A$ receptor α2, α6, β2, γ2 and γ3 subunits in the lung. These results confirm an existence of $GABA_A$ receptor in the adult mouse lung.

In our previous studies, we showed for the first time that mouse brain MECs contain $GABA_A$ receptor, which was stifled by Hcy (42, 43). In the present study, muscimol restored Hcy-suppressed $GABA_A$ receptor expression in mouse brain, aortic and cardiac ECs. This suggests that muscimol may ameliorate the toxic effect of Hcy on EC, such as an impairment of normal cellular function, which leads to apoptosis, lipid peroxidation, enhanced oxidative stress and impaired platelet aggregation (32, 44–47). Furthermore, our finding that $GABA_A$ receptor is expressed in aortic and cardiac MECs suggests that it is present in the endothelium lining of the vasculature of brain, lung, heart and aorta. Thus, $GABA_A$ receptor may have a significant functional role in non-neuronal tissue.

In summary, our data demonstrate that GABA receptor and its subunits are expressed in various non-neuronal tissues in mice, including lung, heart, kidney, stomach and pancreas. Our functional studies show that Hcy inhibits, while muscimol restores, expression of $GABA_A$ receptor in EC. These findings suggest that $GABA_A$ receptor may play a greater role in various physiological or pathological conditions, particularly during HHcy, than it was thought before. A specific gene knockout strategy in mice may accelerate the recognition of the role of $GABA_A$ receptor subtypes as drug targets in neuropathy, cardiopathy and renalpathy.

Acknowledgments

This study was supported in part by NIH grants (HL-71010, HL-88012 and NS-51568 to S.C.T.), American Heart Association (AHA), National SDG (grant 3 0235317N) and NIH (HL-80394 to D.L.) and the AHA post-doctoral training grant (award 3 0625579B to K.S.M.).

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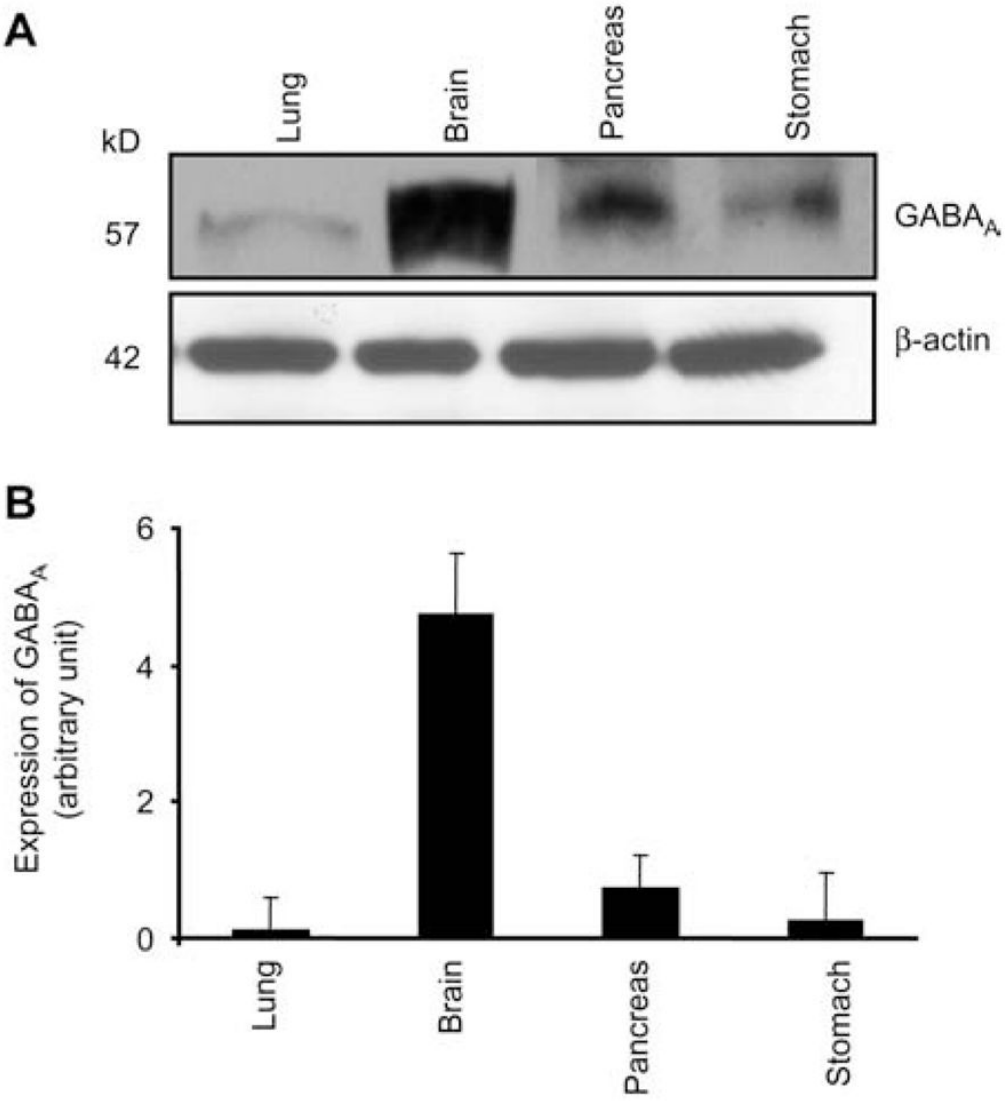

Figure 1.

Expression of the $GABA_A$ receptor in brain (B), lung (L), pancreas (P) and stomach (S). Immunoblot analysis (A) and corresponding densitometry analyses (B). Values are mean ±SEM, n=4 animals from each group. Corresponding β-actin bands are shown.

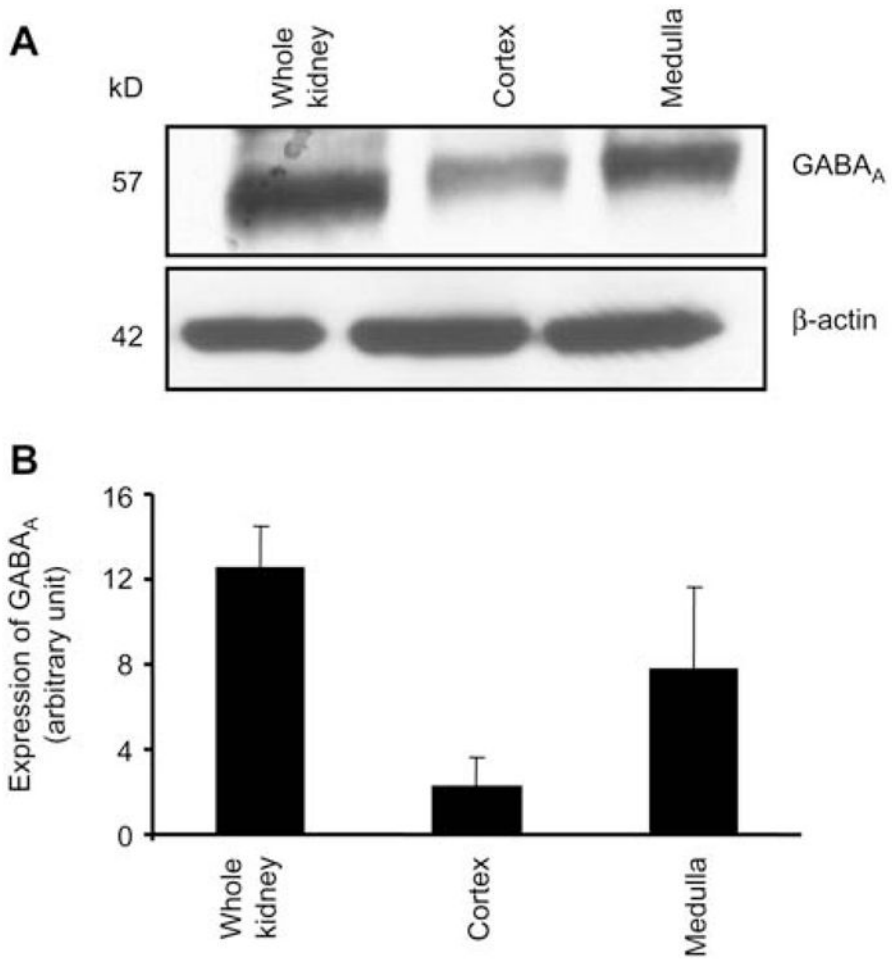

Figure 2.

Expression of the $GABA_A$ receptor in renal system. Whole kidney, medulla and cortex. Western blot analysis of the $GABA_A$ receptor (top) and β-actin (bottom) in renal system (A) and corresponding densitometry analyses (B). Values are mean±SEM, n=4 animals from each group.

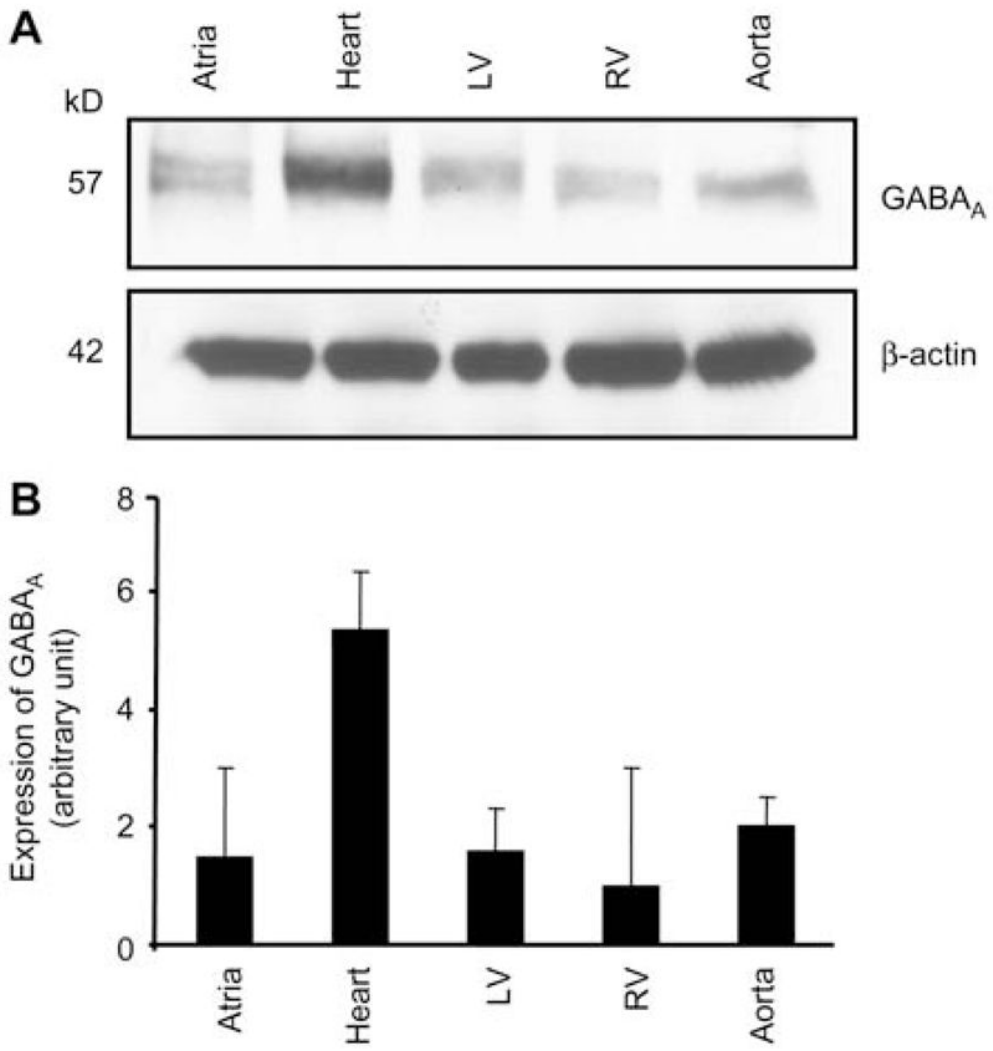

Figure 3.

Expression of the $GABA_A$ receptor in mouse cardiovascular system.

Heart, atrium, left ventricle (LV), right ventricle (RV) and aorta. Western blot analysis of the $GABA_A$ receptor (top) and β-actin (bottom) (A) and corresponding densitometry analyses (B). Values are mean±SEM, n=4 animals from each group.

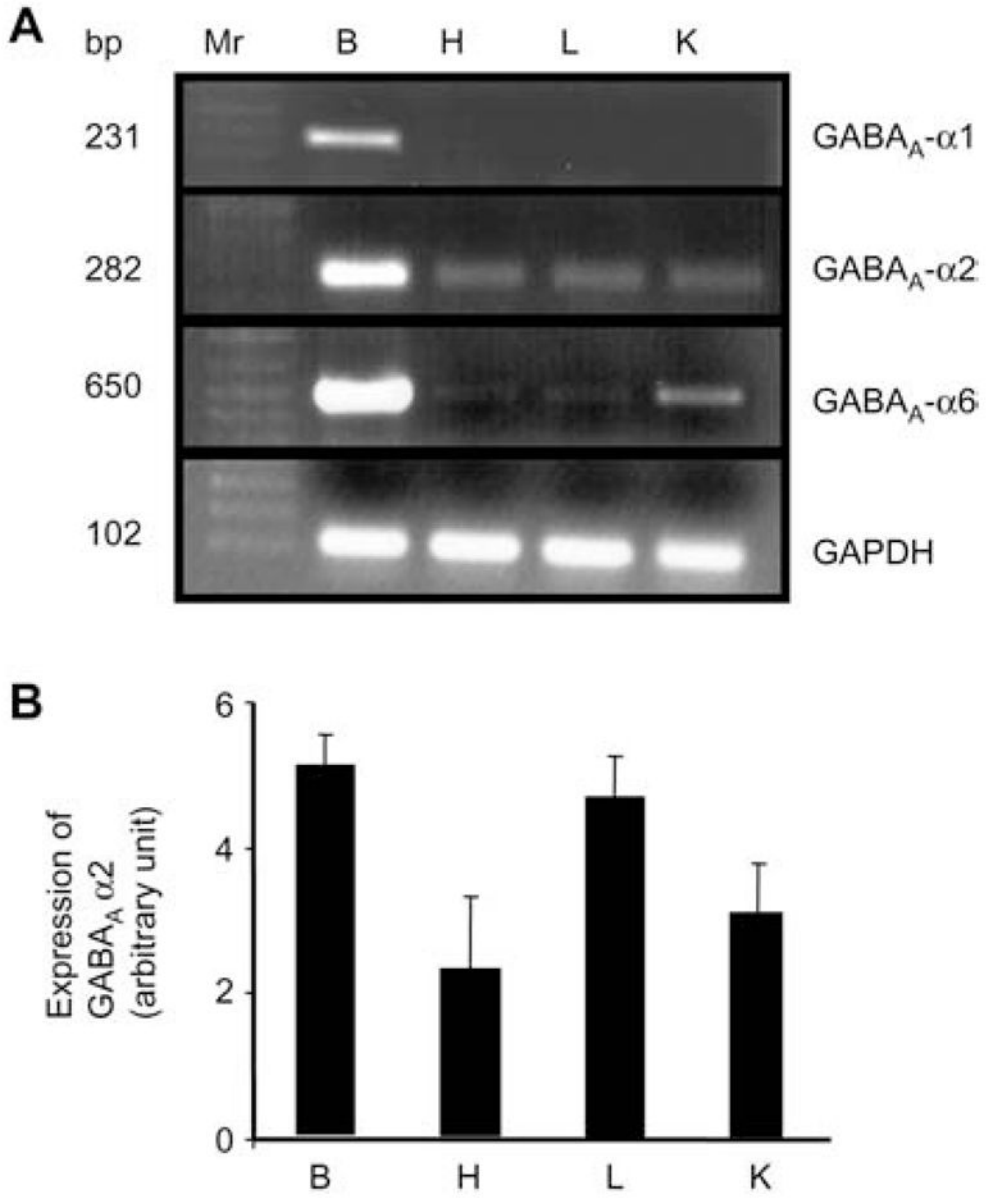

Figure 4.

The mRNA expression of $GABA_A$ receptor α sub-units in brain (B), heart (H), lung (L) and whole kidney (K).

(A) Reverse transcription polymerase chain reaction (RT-PCR) analysis of $GABA_A$-α subunits. Ethidium bromide-stained 1.0% agarose gels containing cDNA amplified from RT RNA using primers for $GABA_A$ subunits (α1–6). A 100-bp DNA ladder (MW) was included at the left of the gel (Lane 1). cDNA bands of the predicted size were obtained for all the above subunits. n=4 animals from each group. (B) Corresponding densitometry analyses (bottom). Values are mean±SEM, n=4 animals from each group.

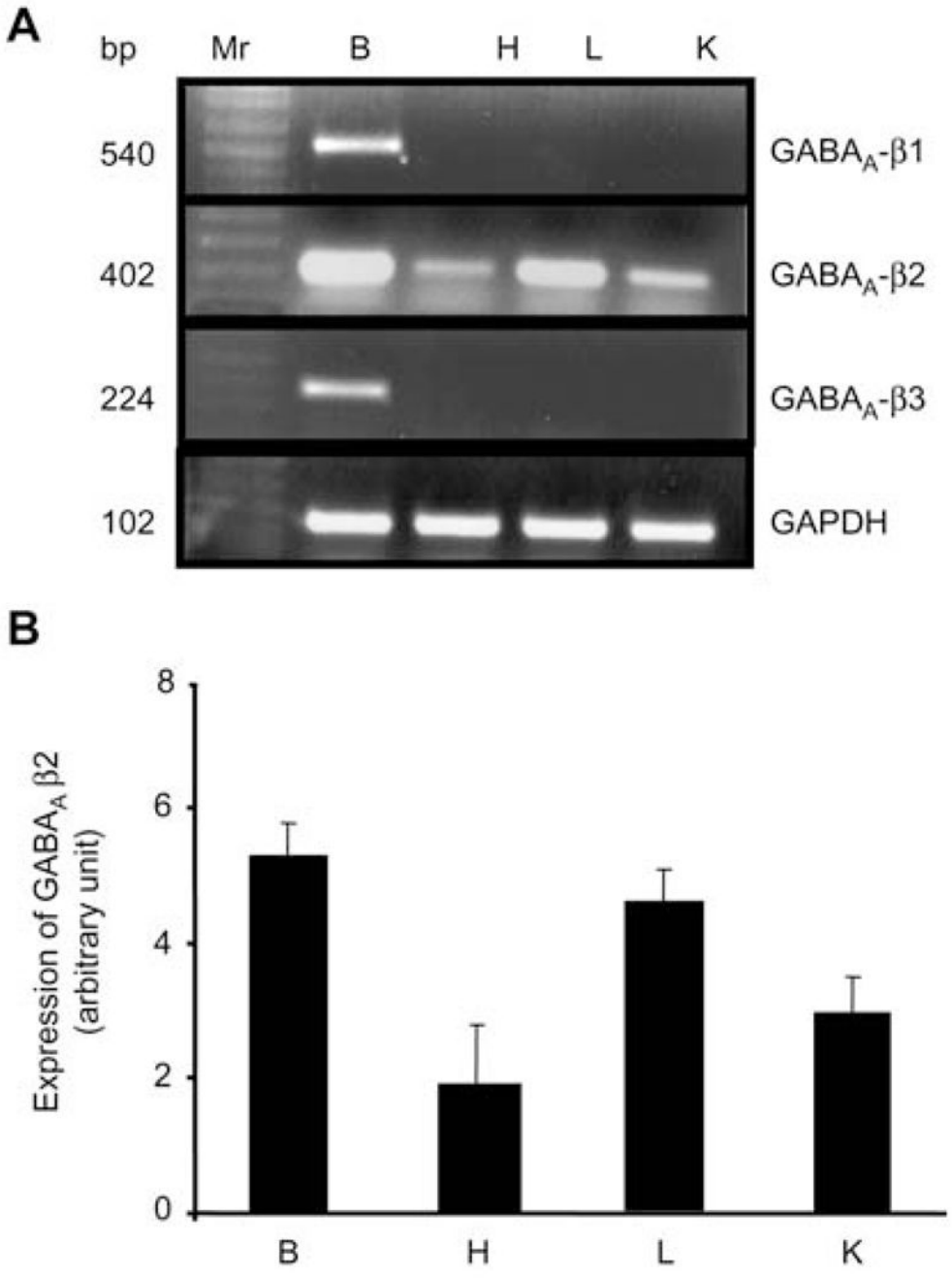

Figure 5.

The mRNA expression of GABA$_A$-β subunits in brain (B), heart (H), lung (L) and whole kidney (K).

(A) Reverse transcription polymerase chain reaction (RT-PCR) analysis of GABA$_A$-β subunits. Ethidium bromide-stained 1.0% agarose gels containing cDNA amplified from RT RNA using primers for GABA$_A$-β1–3. A 100-bp DNA ladder (MW) was included at the left of the gel (Lane 1). cDNA bands of the predicted size were obtained for all the above subunits. n=4 animals from each group. (B) Corresponding densitometry analyses (bottom). Values are mean±SEM, n=4 animals from each group.

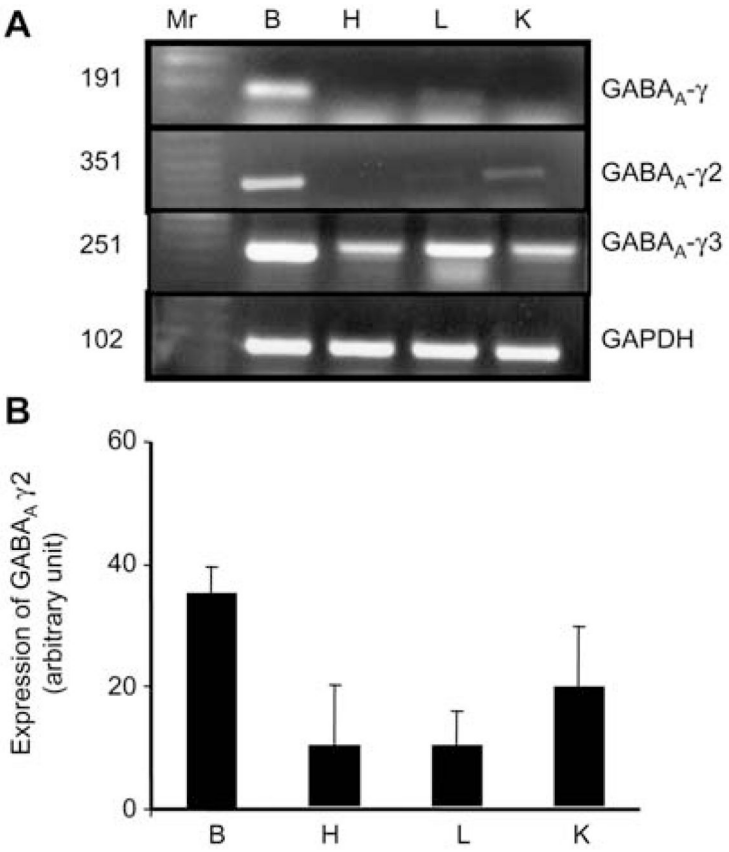

Figure 6.

The mRNA expression of GABA$_A$-γ subunits in brain (B), heart (H), lung (L) and whole kidney (K).

(A) Reverse transcription polymerase chain reaction (RT-PCR) analysis of GABA$_A$-γ subunits. Ethidium bromide-stained 1.0% agarose gels containing cDNA amplified from RT RNA using primers for GABA$_A$-γ1–3. A 100-bp DNA ladder (MW) was included at the left of the gel (Lane 1). cDNA bands of the predicted size were obtained for all the above subunits. n=4 animals from each group. (B) Corresponding densitometry analyses (bottom). Values are mean±SEM, n=4 animals from each group.

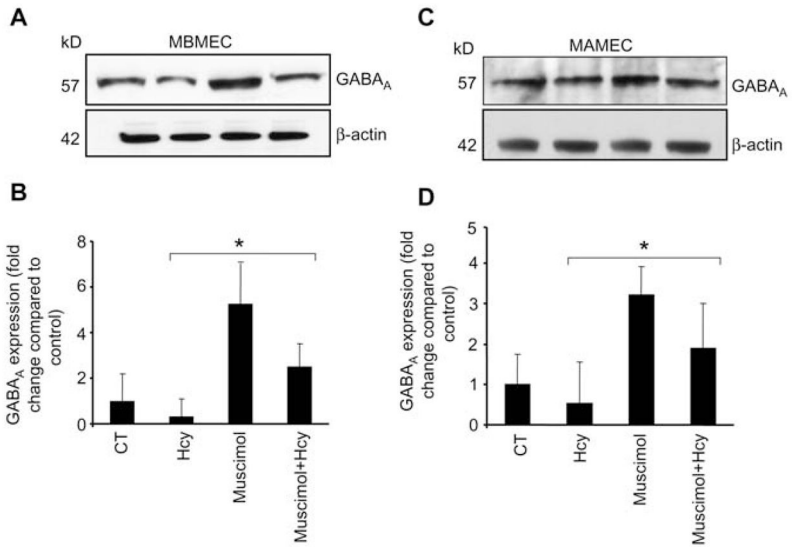

Figure 7.

Western blot analysis showing muscimol-induced expression of $GABA_A$ receptor in cells. Cells (5–7 passages) were plated onto cell culture dishes, grown in complete media and allowed to become 80% confluent. Cells were serum deprived. (A) Mouse brain microvascular endothelial cells (MBMEC) and (C) mouse aorta microvascular endothelial cells (MAMEC) were cultured alone (CT), with 50 μM Hcy, 50 μM muscimol or 50 μM Hcy+50 μM muscimol. After 18 h, equal amounts of cellular protein were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blotted using $GABA_A$ receptor antibody. Corresponding β-actin bands are shown. Accompanying densitometry is shown in (B) and (D), respectively (n=4). *p−0.05 vs. control.

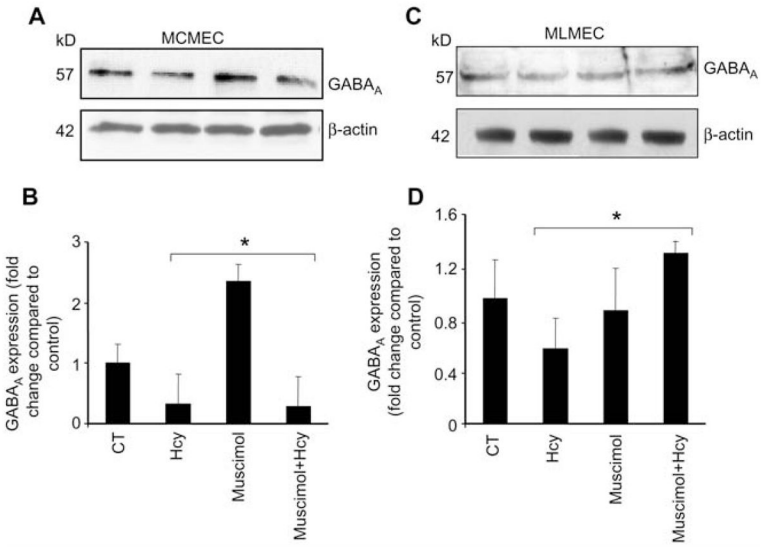

Figure 8.

Western blot analysis showing muscimol-induced expression of $GABA_A$ receptor in cells. Cells (5–7 passages) were plated onto cell culture dishes, grown in complete media and allowed to become 80% confluent. Cells were serum deprived. (A) Mouse cardiac microvascular endothelial cells (MCMEC) and (C) mouse lung microvascular endothelial cells (MLMEC) were cultured alone (CT), with 50 μM Hcy, 50 μM muscimol or 50 μM Hcy +50 μM muscimol. After 18 h, equal amounts of cellular protein were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blotted using $GABA_A$ receptor antibody. Corresponding β-actin bands are shown. Accompanying densitometry is shown in (B) and (D), respectively (n=4). *p−0.05 vs. control.

Table 1

Sequences of oligonucleotide primers used for PCR amplification.

Target	Length, bp	Primer sequence
$GABA_A$-α1	231	5′-CTACAGCAACCAGCTATACCC-3′ 5′-GCTCTCTGTTTAAATACGTGG-3′
$GABA_A$-α2	282	5′-AAGGCTCCGTCATGATACAG-3′ 5′-ACTAACCCCTAATACAGGC-3′
$GABA_A$-α3	418	5′-GACTTGCTTGGTCATGTTGTTGGG-3′ 5′-CAGAGGCCCTGGAGATGAAGAAGA-3′
$GABA_A$-α6	650	5′-CTTCCCTATGGATGGACACG-3′ 5′-GTTGACAGCTGCGAATTCAA-3′
$GABA_A$-β1	540	5′-ATGATGCATCTGCAGCCA-3′ 5′-TGGAGTTCACGTCAGTCA-3′
$GABA_A$-β2	402	5′-GCATGTATGTCTGCAGGA-3′ 5′-CTGACACCTACTTCCTGA-3′
$GABA_A$-β3	224	5′-AGCCAAGGCCAAGAATGATCG-3′ 5′-TGCTTCTGTCTCCCATGTACC-3′
$GABA_A$-γ1	191	5′-TTTCTTACGTGACAGCAATGG-3′ 5′-CATGGGAATGAGAGTGGATCC-3′
$GABA_A$-γ2	351	5′-GCAATGGATCTCTTTGTA-3′ 5′-GTCCATTTTGGCAATGCG-3′
$GABA_A$-γ3	251	5′-TGTCGAAAGCCAACCATCAGG-3′ 5′-GACTTGCACTCCTCATAGCAG-3′