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Review

Gamma-aminobutyric acid (GABA) and cell proliferation: focus on cancer cells

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Summary. In addition to its role in the adult mammalian nervous system as an inhibitory neurotransmitter, γ-aminobutyric acid (GABA) is involved in the proliferation, differentiation, and migration of several kinds of cells including cancer cells. GABA is synthesized predominantly from glutamate by glutamate decarboxylase and exerts its effects via ionotropic $GABA_A$ receptors and/or metabotropic $GABA_B$ receptors. In this review, the current state of knowledge regarding the role of the GABAergic system in peripheral nonneuronal cell proliferation is described, and recent advances in elucidation of the mechanisms leading to cell proliferation are discussed.

Key words: GABA, GABA receptor, Proliferation, Cancer

Introduction

γ-Aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the adult mammalian brain and is thought to be involved in cell proliferation, migration, and in the promotion of cell survival (Barker et al., 1998; Waagepetersen et al., 1999; Ben-Ari, 2002; Owens and Kriegstein, 2002; Ben-Yaakov and Golan, 2003). GABA is also found in many peripheral nonneuronal tissues (Watanabe et al., 2002). GABA exerts its effects through $GABA_A$ and $GABA_B$ receptors; $GABA_A$ receptors (ionotropic) are coupled to chloride ion channels, and $GABA_B$ receptors (metabotropic) are G protein-coupled receptors (GPCRs). GABA receptors are present in many peripheral nonneuronal tissues, indicating that GABA exerts physiologic effects other than neurotransmitter effects in these tissues. In fact, GABA has been shown to be involved in the development of Schwann cells in the peripheral nervous system (Magnaghi et al., 2004) and in the development of the palate (Ding et al., 2004), digestive tract (Gilon et al., 1987b; Wang et al., 2004), pancreas (Gilon et al., 1987a), liver (Minuk et al., 1993, 1997), osteoblasts (Fujimori et al., 2002), chondrocytes (Tamayama et al., 2005), and testicular cells (Geigerseder et al., 2004; Kanbara et al., 2005). GABA is also found in bacteria and plants and is regarded as an intercellular signaling molecule involved in the growth and guidance of pollen tubes in plants (Bouche et al., 2003; Palanivelu et al., 2003; Ma, 2003; Yang, 2003).

Given the function of GABA in these normal tissues and cells, it is intriguing to consider the potential function of GABA in cancer cells. Recently, we reported that GABA participates in the metastasis of prostate cancer cells (Azuma et al., 2003) and that GABA is highly expressed in colonic tumor cells (Maemura et al., 2003). In this review, we address the role of GABA in cell proliferation and migration, including that of cancer cells.

Metabolic pathways of GABA

Detailed metabolic pathways of GABA have been described elsewhere (Watanabe et al., 2002); therefore, only the main metabolic pathways will be described here. The major biosynthetic pathway of GABA involves decarboxylation of glutamate by glutamate decarboxylase (GAD). GABA catabolism is catalyzed by GABA transaminase. GAD is the rate-limiting enzyme in GABA synthesis. Mammalian species express two isoforms of GAD, GAD65 and GAD67, which are the products of two distinct genes. The GAD65 gene is located on chromosome 10, and the GAD67 gene is located on chromosome 2 in humans. The GAD65 and GAD67 genes are derived from a common ancestral gene. Because this is the only known case of two genes encoding an enzyme required for the synthesis of a neurotransmitter, it is speculated that the two GAD isoforms have distinct physiologic roles.

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GABA receptors

GABA functions by binding to its specific receptors, $GABA_A$ and $GABA_B$. The $GABA_A$ receptor is a pentamer comprised of various subunits: α1-6, ß1-3, γ1-3, δ, ε, π, and θ. Each subunit possesses four hydrophobic membrane-spanning domains. Three ρ subunits (ρ1-3) have also been reported, and receptor assemblies derived from r subunits are classified as $GABA_C$ receptors (Bolmann, 2000). Because $GABA_A$ receptors are pentameric assemblies of various subunits, a very large number of receptor subtypes can be formed. Studies have shown that functional $GABA_A$ receptors contain at least one α, one ß, and one γ subunit, and δ, ε, π, and θ subunits are thought to be assembled into $GABA_A$ receptors in place of γ subunits (McKerman and Whiting, 1996). The ε subunit is only found in the liver (Erlitzki et al., 2000), and the π subunit is found in the uterus, prostate gland, thymus, and lung (Hedblom and Kirkness, 1997).

$GABA_B$ receptors belong to the GPCR family, members of which possess seven membrane-spanning domains. $GABA_B$ receptors are heterodimers of two receptor subunits, $GABA_B$ R1 and $GABA_B$ R2. Each subunit has an extracellular N-terminal domain and an intracellular C-terminal domain. The subunits interact via intracellular coiled-coil domains near the C terminus. $GABA_B$ receptors are coupled to Gi/Go (Bormann, 2000), and activation of $GABA_B$ receptors decreases cAMP accumulation by inhibiting the activity of adenylate cyclase. However, although cAMP concentration increased by treatment with forskolin is decreased by activation of $GABA_B$ receptors, activation of $GABA_B$ receptors increases the cAMP accumulation induced by isoproterenol, an agonist of ß-adrenergic receptors coupled to G_s (Hedblom and Kirkness, 1997).

GABA modulates pollen tube growth in plants

GABA was first discovered in plants (Steward et al., 1949) and is conserved from bacteria to yeast to vertebrates. GABA has recently been reported to be essential for the growth and guidance of plant pollen tubes (Bouché et al., 2003; Palanivelu et al., 2003; Ma, 2003), and similarities between the growth of neuronal axons and pollen tubes has been suggested (Bouché et al., 2003). Transgenic plants ectopically expressing constitutively active GAD have a higher GABA content and a lower glutamate content, resulting in abnormal growth and development (Baum et al., 1996). Plants with a disruption of one of the five GAD genes show less well-developed roots than those of wild-type plants (Bouché et al., 2003).

GABA mediates proliferation of neuronal cells

In 1978, GABA was found to promote synaptogenesis in the superior cervical ganglion of the adult rat (Wolff et al., 1978). It has since been shown that GABA can influence neuronal differentiation (Meier et al., 1991). At the time, the mechanism underlying the trophic actions of GABA was obscure, but they appeared to be mediated by the depolarization of immature neurons via activation of $GABA_A$ receptors. This depolarization is sufficient to activate voltage-gated calcium channels (Lin et al., 1994; Leinekugel et al., 1995). Bromodeoxyuridine (BrdU) studies of the effect of GABA on neuronal proliferation showed opposing effects of stimulation and inhibition (LoTurco et al., 1995; Borodinsky and Fisuman, 1998; Cui and Bulleit, 1998; Haydar et al., 2000). Thus, the effect of GABA on neuronal proliferation may be cell type- and region-specific. With respect to neuron migration, studies have shown that GABA can influence the migration of embryonic cortical neurons via GAB_A and $GABA_B$ receptors (Behar et al., 1996, 2000). With respect to differentiation, GABA may promote neurite outgrowth and maturation of cultured embryonic hippocampal and neocortical neurons (Barbin et al., 1993; Maric et al., 2001). More detailed discussions may be found in recent reviews (Owence and Kriegstein, 2002; Ben-Ari, 2002; Takayama and Inoue, 2004).

GABA regulates proliferation of peripheral nonneuronal cells

There has been little research on the participation of the GABAergic system on the proliferation of normal nonneuronal cells. We reported the distribution of GABA in growth plate chondrocytes with ^{14}C-GABA injected into mouse tail vein (Kuroda et al., 2000) and also reported the expression of GABA and GAD in the proliferative and hypertrophic zones of rat tibial growth plate (Tamayama et al., 2001). Further investigation revealed the expression of both $GABA_A$ and $GABA_B$ receptor subunit mRNAs and proteins in rat growth plate chondrocytes (Tamayama et al., 2005). Studies with the ATDC5 mouse chondrogenic cell line showed that GABA contributes to cell proliferation via activation of $GABA_A$ and $GABA_B$ receptors (Tamayama et al., 2005).

Studies of GAD65/67 double-mutant mice, which completely lack GAB_A (Ji et al., 1999), and GAD67-deficient mice (Ding et al., 2004) showed that these mice are born with a cleft palate. The requirement for GABA in normal development of the palate has also been shown in $GABA_A$ receptor ß3 subunit-knockout mice (Culiat et al., 1993, 1995; Homanics et al., 1997). Cleft palate was also observed in the offspring of pregnant mice treated with $GABA_A$ receptor antagonist or GAD inhibitor (Ding et al., 2004). These results indicate that GABA is required for palatogenesis.

In the peripheral nervous system, Schwann cells prepared from the sciatic nerve of 3-day-old rats express $GABA_B$ receptors, and addition of the $GABA_B$ receptor agonist baclofen to the incubation medium inhibits Schwann cell proliferation, suggesting that GABA can influence cell proliferation (Magnaghi et al., 2004). In the testis, GABA is expressed in the acrosome, and

$GABA_BR1$ and $GABA_BR2$ subunits are expressed in the acrosomal membrane during spermiogenesis. These results indicate that the GABAergic system is involved in the regulation of spermiogenesis (Kanbara et al., 2005). In addition, proliferation of Leydig cells is stimulated by GABA via activation of $GABA_A$ receptors (Geigerseder et al., 2004). Because mature Leydig cells express GAD and $GABA_A$ and $GABA_B$ receptors, it is assumed that GAB_A acts in an auto/paracrine manner *in vivo*.

In the digestive system, GABA uptake in rat pancreas from 15.5 days in utero to 105 days after birth was investigated by autoradiography, and GABA content was measured by liquid chromatography (Gilon et al., 1987b). This study showed that both GABA uptake and content were significantly greater in the early postnatal period than in later stages. Thus GABA may be involved in the development of the pancreas. Similar results were obtained in rat duodenum (Gilon et al., 1987a). In rat jejunum, uptake of ÇRH-thymidine and immunostaining of proliferating cell nuclear antigen are limited to the jejunal cripts, and GABA and GAD65 immunoreactivities are distributed in the jejunal villi, suggesting that GABA is involved in epithelial cell differentiation in rat jejunum (Wang et al., 2004). However, these results also assumed that GABA has an inhibitory effect on epithelial cell proliferation. Expression of $GABA_A$ receptors has also been reported in rat and human liver (Minuk et al., 1987; Erlitzki et al., 2000), and GABA exerts an inhibitory effect via $GABA_A$ receptors on hepatic regeneration after partial hepatectomy or liver injury (Minuk and Gauthier, 1993; Zhang et al., 1996; Kaita et al., 1998). Thus, opposing effects of GABA on cell growth occurs in peripheral nonneuronal cells as well as in neuronal cells.

Function of GABA on cancer cell proliferation

Given that GABA participates in the proliferation of various normal cell types, a role in cancer cell proliferation can be considered. Increased GAD activity and GABA content have been reported in colon cancer (Kleinrok et al., 1998; Wang et al., 2000; Maemura et al., 2003), breast cancer (Mazurkiewicz et al., 1999; Opolski et al., 2000), prostate cancer (Azuma et al., 2003; Hu et al., 2001), gastric cancer (Matuszek et al., 2001), and glioma (Bianchi et al., 2004). Furthermore, GABA receptor mRNAs and proteins are increased in neuroblastoma (Roberts et al., 2004), liver cancer (Biju et al., 2002), and breast cancer (Jiang et al., 2002). In N-nitrosodiethylamine-induced neoplasia in the rat liver, $GABA_B$ receptors were increased and the $GABA_B$ receptor agonist baclofen increased EGF-mediated DNA synthesis in hepatocytes (Biju et al., 2002). In contrast, increased $GABA_A$ receptor activity inhibits expression of α-fetoprotein mRNA and proliferation of the HepG2 human hepatocellular carcinoma cell line (Zhang et al., 2000). The incidence of rat gastric cancers induced by nitrosoguanidine was decreased by administration of GABA or baclofen but not the $GABA_A$ receptor agonist muscimol (Tatsuta et al., 1990). In addition, GABA and baclofen decreased the BrdU labeling index of the gastric antral mucosa. In the colon, baclofen decreased the incidence of carcinogenesis induced by azoxymethane (Tatsuta et al., 1992).

Modulatory role of GABA in cellular motility

GABA has been reported to induce the migration of dissociated embryonic cortical neurons (Behar et al., 1996, 2000). Chemokine and catecholamine receptors, which are GPCRs, regulate the migration of leukocytes and tumor cells (Entschladen et al., 2002). The effect of $GABA_B$ receptors on migration has also been studied in cancer cells (Joseph et al., 2002); GABA reduced the migratory activity of SW480 colon carcinoma cells by activating $GABA_B$ receptors and decreasing cAMP concentration. However, in prostate cancer patients with metastasis and lymph node metastasis, expression of GAD67 and GABA was markedly greater than that in patients without metastasis (Azuma et al., 2003). In addition to GAD67 and GABA, matrix metalloproteinase (MMP) expression was substantially elevated in prostate cancer patients with metastasis and lymph node metastasis. MMP expression was induced by activation of $GABA_B$ receptors and increased the invasive ability of cancer cells. In human breast cancer cells, migration was stimulated by the $GABA_A$ receptor agonist propofol (Garib et al., 2002).

Effect of GABA signal transduction via $GABA_A$ receptors on cell proliferation

Activation of GABAA receptors by GABA opens Cl^- channels. Consequently, GABA induces hyperpolarization when the intracellular Cl^- concentration is relatively low compared to the extracellular $C1^-$ concentration, whereas depolarization occurs when the intracellular $C1^-$ concentration is relatively high. In immature neurons, activation of $GABA_A$ receptors produces depolarization (Wang et al., 2005) sufficient to increase the intracellular Ca^{2+} concentration by activation of voltage-gated Ca^{2+} channels (Leinekugel et al., 1995; Gao and van del Pol, 2001). By inducing depolarization and elevated intracellular Ca^{2+}, GABA acts in an excitatory manner to influence neurogenesis and synaptogenesis (Fiszman et al., 1999; Ashwort and Bolsover, 2002; Deisseroth et al., 2004). Increased intracellular Ca^{2+} activates the small guanine nucleotide-binding protein Ras and stimulates the mitogen-activated protein kinase (MAPK) cascade in PC12 cells (Rosen et al., 1994) and immature rat cerebellar granule cells (Fiszman et al., 1999) (Fig. 1).

Effect of GABA signal transduction via $GABA_B$ receptors on cell proliferation

There are currently no reports describing the signal

transduction system involved in $GABA_B$ receptor activation of cell proliferation. However, the molecular mechanisms involved in GPCR regulation of cell proliferation have been the subject of intensive investigation. Based on the results of these studies, we may identify possible signal transduction pathways of $GABA_B$ receptors. GPCR signal transduction pathways leading to cell proliferation predominantly involve receptor tyrosine kinases. It is well known that activation of receptor tyrosine kinases promote development and proliferation of many types of cancer cells (Yarden, 2001). Growth factors such as epidermal growth factor (EGF), transforming growth factor-α (TGF-α), and fibroblast growth factor (FGF) activate specific receptor tyrosine kinases, inducing proliferation and differentiation by various signal transduction pathways. The EGF receptor is the most characterized receptor tyrosine kinase; it is a transmembrane receptor composed of an extracellular ligand-binding domain and a cytoplasmic region with enzymatic activity (Ullrich and Schlessinger, 1990). Binding of ligand, such as EGF or TGF-α, induces receptor dimerization and activation of the intracellular kinase domain on each receptor, resulting in phosphorylation of tyrosine residues on each receptor and the formation of docking sites for signaling complexes composed of cytoplasmic enzymes and adaptor proteins. The subsequent dissociation of these signaling complexes releases activated effector and adaptor proteins into the cytoplasm, where they activate downstream signaling pathways including the MAPK pathway, thereby mediating cellular responses (Yarden, 2001) (Fig. 2). Several recent studies have documented cross-communication between GPCRs and the EGF receptor. As shown in Fig. 3, the GCPR agonists endothelin, lysophosphatidic acid (LPA), and thrombin stimulate proliferation of Rat-1 fibroblasts by activating the MAPK pathway (Daub et al., 1996). Subsequent studies have reported such cross-talk in other cell types including human keratinocytes, COS-7 cells, PC12 cells, and embryonic kidney cells (Daub et al., 1997; Zwick et al., 1999; Alderton et al., 2001). These findings indicate that many extracellular signals leading to cell proliferation are transmitted by GPCRs in collaboration with receptor tyrosine kinases, particularly the EGF receptor. GPCRs coupled to Gi or Gq proteins can transactivate tyrosine kinases (Crespo et al., 1994; Daub et al., 1997).

In contrast, various GCPR agonists, including vasopressin, norepinephrine, prostaglandin $F_{2\alpha}$, and angiotensin II, increase the expression of c-Myc and activating transcription factor 3/liver regeneration factor-1 (ATF3/LRF-1), transcription factors that induce cell proliferation in the absence of EGF receptor transactivation in rat hepatocytes (Nilssen et al., 2004). Interestingly, there is evidence that in colonic epithelial cells, binding of substance P to the GCPR neurokinin-1 receptor activates MMPs that cleave the membrane-bound precursor pro-TGF-α, leading to shedding of mature TGF-α into the extracellular space. Mature TGF-α binds and activates the EGF receptor, leading to MAPK signaling followed by cell proliferation (Koon et al., 2004) (Fig. 4). The neurokinin-1 receptor couples to Gi, as does the $GABA_B$ receptor. MMPs activated by GPCR stimulation are capable of inducing shedding of another EGF receptor ligand, heparin-binding EGF-like growth factor (HB-EGF), leading to EGR receptor activation (Yan et al., 2002; Prenzel et al., 1999). We

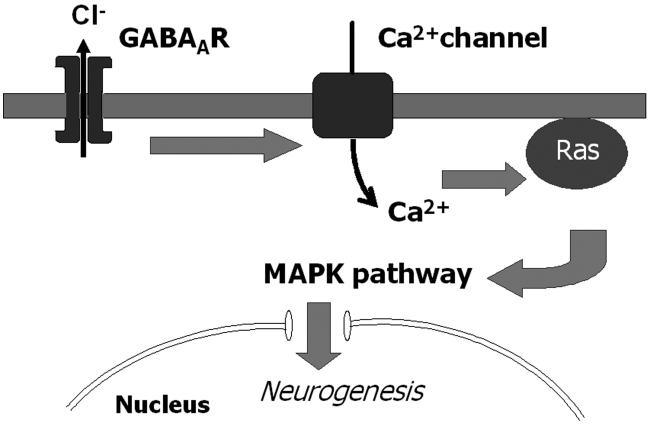

Fig. 1. Signal transduction via $GABA_A$ receptors leads to neurogenesis. In response to membrane depolarization, calcium influx through L-type calcium channels leads to activation of the small guanine nucleotide-binding protein Ras. Ras activation leads to activation of the mitogen-activated protein kinase (MAPK) pathway.

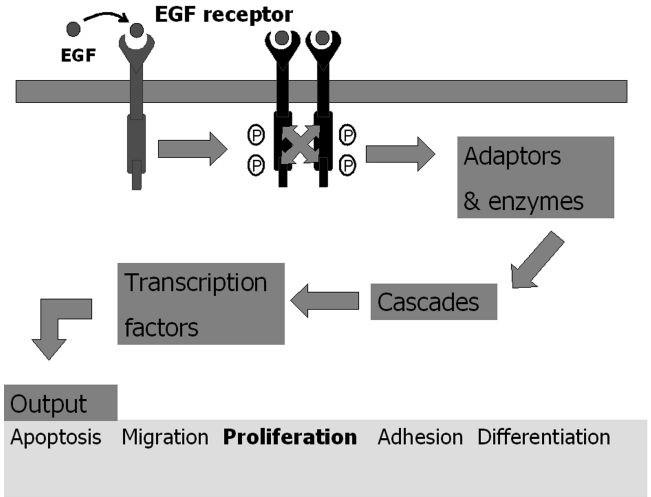

Fig. 2. Signal transduction via epidermal growth factor (EGF) receptors leads to various cellular responses. EGF receptor is a receptor tyrosine kinase. A number of different ligands, including EGF-like molecules and transforming growth factor (TGF)-α, dimerize and activate the receptor. A transient signaling complex composed of effector and adaptor proteins is then assembled. Dissociation of this complex activates many signal transduction cascades including the MAPK pathway.

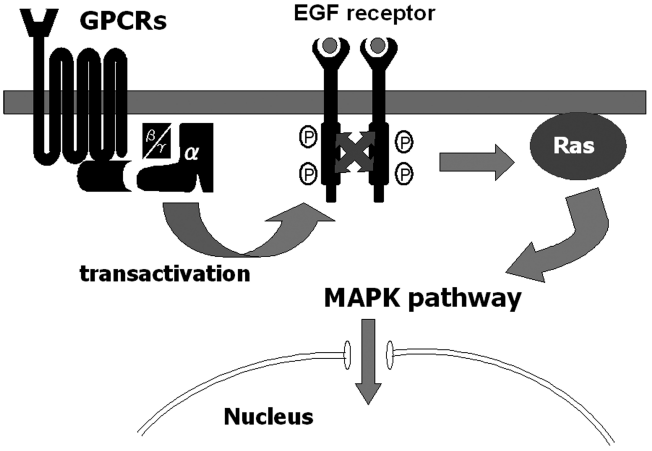

Fig. 3. Transactivation of the EGF receptor by G protein-coupled receptors (GPCRs). Several agonists, including lysophosphatidic acid (LPA), endothelin-1, and thrombin, activate GPCRs, which leads to tyrosine phosphorylation and activation of the EGF receptor.

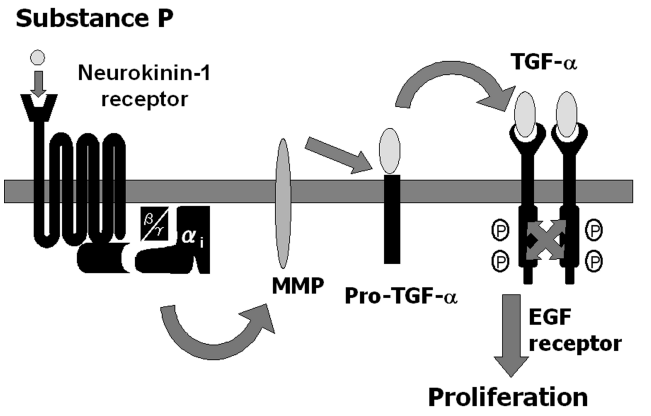

Fig. 4. Schematic of EGF receptor transactivation in response to GPCR stimulation. Binding of the agonist substance P to the GPCR neurokinin-1 receptor, which is coupled to Gi, stimulates the matrix metalloproteinase-dependent release of the preformed EGF receptor ligand, transforming growth factor (TGF)-α. Active TGF-α stimulates EGF receptors, leading to Ras-dependent activation of the MAPK pathway.

reported that in prostate cancer cells, MMPs levels are increased by activation of GABAB receptors (Azuma et al., 2003). These findings led us to hypothesize that GABA induces cell proliferation via activation of the $GABA_B$ receptor and MMPs, followed by enhancement of the effect of mitogens such as TGF-α and HB-EGF.

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References

Alderton F., Sambi B., Tate R., Pyne N.J. and Pyne S. (2001). Assessment of agonism at G-protein coupled receptors by phosphatidic acid and lysophosphatidic acid in human embryonic kidney 293 cells. Brit. J. Pharmacol. 134, 6-9.

Ashworth R. and Bolsover S.R. (2002). Spontaneous activity-independent intracellular calcium signals in the developing spinal cord of the zebra fish embryo. Brain Res. Dev. Brain Res.139, 131-137.

Azuma H., Inamoto T., Sakamoto T., Kiyama S., Ubai T., Shinohara Y., Maemura K., Tsuji M., Segawa N., Masuda H., Takahara K., Katsuoka Y. and Watanabe M. (2003). γ-aminobutyric acid as a promoting factor of cancer metastasis; induction of matrix metalloproteinase production is potentially its underlying mechanism. Cancer Res. 63, 8090-8098.

Barbin G., Pollard H., Gaiarsa J.L. and Ben-Ari Y. (1993). Involvement of GABAA receptors in the outgrowth of cultured hippocampal neurons. Neurosci. Lett. 152, 150-154.

Barker J.L., Behar T., Li Y.X., Liu Q.Y., Ma W., Maric D., Maric I., Schaffner A.E., Serafini R., Smith S.V., Somogyi R., Vautrin J.Y., Wen X.L. and Xian H. (1998). GABAergic cells and signals in CNS development. Perspect. Dev. Neurobiol. 5, 305-322.

Baum G., Lev-Yadun S., Fridmann Y., Arazi T., Katsnelson H., Zik M. and Fromm H. (1996). Calmodulin binding to glutamate decarboxilase is required for regulation of glutamate and GABA metabolism and normal development in plants. EMBO J. 15, 2988-2996.

Behar T.N., Li Y.X., Tran H.T., Ma W., Dunlap V., Scott C. and Barker J.L. (1996). GABA stimulates chemotaxis and chemokinesis of embryonic cortical neurons via calcium-dependent mechanisms. J. Neurosci. 16, 1808-1818.

Behar T.N., Schaffner A.E., Scott C.A., Greene C.L. and Barker J.L. (2000).GABA receptor antagonists modulate postmitotic cell migration in slice cultures of embryonic rat cortex. Cereb. Cortex 10, 899-909.

Ben-Ari Y. (2002). Excitatory actions of gaba during development: the nature of the nurture. Nat. Rev. Neurosci. 3, 728-739.

Ben-Yaakov G. and Golan H. (2003). Cell proliferation in response to GABA in postnatal hippocampal slice culture. Int. J. Dev. Neurosci. 21, 153-157.

Bianchi L., De Micheli E., Bricolo A., Ballini C., Fattori M., Venturi C., Pedata F., Tipton K.F. and Della Corte L. (2004). Extracellular levels of amino acids and choline in human high grade gliomas: an intraoperative microdialysis study. Neurochem. Res. 29, 325-334.

Biju M.P., Pyroja S., Rajeshkumar N.V. and Paulose C.S. (2002). Enhanced GABA(B) receptor in neoplastic rat liver: induction of DNA synthesis by baclofen in hepatocyte cultures. J. Biochem. Mol. Biol. Biophys. 6, 209-214.

Bolmann J. (2000). The 'ABC' of GABA receptors. Trends. Pharmacol. Sci. 21, 16-19.

Borodinsky L.N. and Fiszman M.L. (1998). Extracellular potassium concentration regulates proliferation of immature cerebellar granule cells. Brain Res. Dev. Brain Res. 107, 43-48.

Bouché N., Lacombe B. and Fromm H. (2003). GABA signaling: a conserved and ubiquitous mechanism. Trends Cell. Biol. 13, 607-610.

Crespo P., Xu N., Simonds W.F. and Gutkind J.S. (1994) Ras-dependent activation of MAP kinase pathway mediated by G-protein bg subunits. Nature 369, 418-420.

Cui H. and Bulleit R.F. (1998). Potassium chloride inhibits proliferation of cerebellar granule neuron progenitors. Brain Res. Dev. Brain Res. 106, 129-135.

Culiat C.T., Stubbs L.J., Nicholls R.D., Montgomery C.S., Russell L.B., Johnson D.K. and Rinchik E.M. (1993). Concordance between isolated cleft palate in mice and alterations within a region including the gene encoding the ß3 subunit of the type A γ-aminobutyric acid receptor. Proc. Natl. Acad. Sci. USA 90, 5105-5109.

Culiat C.T., Stubbs L.J., Woychik R.P., Russell L.B., Johnson D.K. and Rinchik E.M. (1995). Deficiency of the ß3 subunit of the type A γ-aminobutyric acid receptor causes cleft palate in mice. Nat. Genet. 11, 344-346.

Daub H., Weiss F.U., Wallasch C. and Ullrich A. (1996). Role of transactivation of the EGF receptor in signaling by G-protein-coupled receptors. Nature 379, 557-560.

Daub H., Wallasch C., Lankenau A., Herrlich A. and Ullrich A. (1997). Signal characteristics of G protein-transactivated EGF receptor. EMBO J. 16, 7032-7044.

Deisseroth K., Singla S., Toda H., Monje M., Palmer T.D. and Malenka R.C. (2004). Excitation-neurogenesis coupling in adult neural stem/progenitor cells. Neuron 42, 535-552.

Ding R., Tsunekawa N. and Obata K. (2004). Cleft palate by picrotoxin or 3-MP and palatal shelf elevation in GABA-deficient mice. Neurotoxicol. Teratol. 26, 587-592.

Entschladen F., Lang K., Drell T.L., Joseph J. and Zaenker K.S. (2002). Neurotransmitters are regulators for the migration of tumor cells and leukocytes. Cancer Immunol. Immunother. 51, 467-482.

Erlitzki R., Gong Y., Zhang M. and Minuk G. (2000). Identification of g-aminobutyric acid receptor subunit types in human and rat liver. Am. J. Physiol. Gastroint. Liver Physiol. 279, G733-G739.

Fiszman M.L., Borodinski L.N. and Neale J.H. (1999). GABA induces proliferation of immature cerebellar granule cells growth *in vitro*. Dev. Brain. Res. 115, 1-18.

Fujimori S., Hinoi E. and Yoneda Y. (2002). Functional $GABA_B$ receptors expressed in cultured calvarial osteoblasts. Biochem. Biophys. Res. Commun. 293, 1445-1452.

Gao X.B. and van den Pol A.N. (2001). GABA, not glutamate, a primary transmitter driving action potentials in developing hypothalamic neurons. J. Neurophysiol. 85, 425-434.

Garib V., Niggemann B., Zanker K.S., Brandt L. and Kubens B.S. (2002). Influence of non-volatile anesthetics on the migration behavior of the human breast cancer cell line MDA-MB-468. Acta Anaesthesiol. Scand. 46, 836-844.

Geigerseder C., Doepner R.F., Thalhammer A., Krieger A. and Mayerhofer A. (2004). Stimulation of TM3 Leydig cell proliferation via GABA(A) receptors: a new role for testicular GABA. Reprod. Biol. Endocrinol. 2, 13.

Gilon P., Remacle C., Janssens de Varebeke P., Pauwels G. and Hoet J.J. (1987a). GABA content and localisation of high-affinity GABA uptake during the development of the rat pancreas. Cell. Mol. Biol. 33, 573-585.

Gilon P., Reusens-Billen B., Remacle C., Janssens de Varebeke P., Pauwels G. and Hoet J.J. (1987b). Localization of high-affinity GABA uptake and GABA content in the rat duodenum during development. Cell Tissue. Res. 249, 593-600.

Haydar T.F., Wang F., Schwartz M.L. and Rakic P. (2000). Differential modulation of proliferation in the neocortical ventricular and subventricular zones. J. Neurosci. 20, 5764-5774.

Hedblom E. and Kirkness E.F. (1997). A novel class of GABAA receptor subunit in tissues of the reproductive system. J. Biol. Chem. 272, 15346-15350.

Homanics G.E., DeLorey T.M., Firestone L.L., Quinlan J.J., Handforth A., Harrison N.L., Krasowski M.D., Rick C.E., Korpi E.R., Makela R., Brilliant M.H., Hagiwara N., Ferguson C., Snyder K. and Olsen R.W. (1997). Mice devoid of gamma-aminobutyrate type A receptor beta3 subunit have epilepsy, cleft palate, and hypersensitive behavior. Proc. Natl. Acad. Sci. USA 94, 4143-4148.

Hu Y., Ippolito J.E., Garabedian E.M., Humphrey P.A. and Gordon J.I. (2001). Molecular characterization of a metastatic neuroendocrine cell cancer arising in the prostates of transgenic mice. J. Biol. Chem. 277, 44462-44474.

Ji F.Y., Kanbara N. and Obata K. (1999). GABA and histogenesis in fetal and neonatal mouse brain lacking both isoform of glutamic acid decarboxylase. Neurosci. Res. 33, 187-194.

Jiang Y., Harlocker S.L., Molesh D.A., Dillon D.C., Stolk J.A., Houghton R.L., Repasky E.A., Badaro R., Reed S.G. and Xu J. (2002). Discovery of differentially expressed genes in human breast cancer using subtracted cDNA libraries and cDNA microarrays. Oncogene 21, 2270-2282.

Joseph J., Niggemann B., Zaenker K.S. and Entschladen F. (2002). The neurotransmitter gamma-aminobutyric acid is an inhibitory regulator for the migration of SW 480 colon carcinoma cells. Cancer Res. 62, 6467-6469.

Kaita K.D.E., Assy N., Gauthier T., Zhang M., Meyers A.F. and Minuk G.Y. (1998). The beneficial effects of ciprofloxacin on survival and hepatic regenerative activity in a rat model of fulminant hepatic failure. Hepatology 27, 533-536.

Kanbara K., Okamoto K., Nomura S., Kaneko T., Shigemoto R., Azuma H., Katsuoka Y. and Watanabe M. (2005). Cellular localization of GABA and GABAB receptor subunit proteins during spermatogenesis in rat testis. J. Androl. 26, 485-493.

Kleinrok Z., Matuszek M., Jesipowicz J., Matuszek B., Opolski A. and Radzikowski C. (1998). GABA content and GAD activity in colon tumors taken from patients with colon cancer or from xenografted human colon cancer cells growing as s.c. tumors in athymic nu/nu mice. J. Physiol. Pharmacol. 49, 303-310.

Koon H.W., Zhao D., Na X., Moyer M.P. and Pothoulakis C. (2004). Metalloproteinases and transforming growth factor-alpha mediate substance P-induced mitogen-activated protein kinase activation and proliferation in human colonocytes. J. Biol. Chem. 279, 45519-45527.

Kuroda E., Watanabe M., Tamayama T. and Shimada M. (2000). . Autoradiographic distribution of radioactivity from ^{14}C-GABA in the mouse. Microsc. Res. Tech. 48, 116-126.

Leinekugel X., Tseeb V., Ben-Ari Y. and Bregestovski P. (1995). Synaptic $GABA_A$ activation induces Ca^{2+} rise in pyramidal cells and interneurons from rat neonatal hippocampal slices. J. Physiol. 487, 319-329.

Lin M.H., Takahashi M.P., Takahashi Y. and Tsumoto T. (1994). Intracellular calcium increase induced by GABA in visual cortex of fetal and neonatal rats and its disappearance with development. Neurosci. Res. 20, 85-94.

LoTurco J.J., Owens D.F., Heath M.J.S., Davis M.B.E. and Kriegstein A.R. (1995) GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. Neuron 15, 1287-1298.

Ma H. (2003). Plant reproduction: GABA gradient, guidance and growth. Curr. Biol. 13, R834-836.

Maemura K., Yamauchi H., Hayasaki H., Kanbara K., Tamayama T., Hirata I. and Watanabe M. (2003). γ-Amino-butyric acid immunoreactivity in intramucosal colonic tumors. J. Gastroenterol. Hepatol. 18, 1089-1094.

Magnaghi V., Ballabio M., Cavarretta I.T.R., Froestl W., Lambert J.J., Zucchi I. and Melcangi R.C. (2004). GABAB receptors in Schwann cells influence proliferation and myelin protein expression. Eur. J. Neurosci. 19, 2641-2649.

Maric D., Liu Q.Y., Maric I., Chaudry S., Chang Y.H., Smith S.V., Sieghart W., Fritschy J.M. and Barker J.L. (2001). GABA expression dominates neuronal lineage progression in the embryonic rat neocortex and facilitates neurite outgrowth via GABA(A) autoreceptor/Cl- channels. J. Neurosci. 21, 2343-2360.

Matuszek M., Jesipowicz M. and Kleinrok Z. (2001). GABA content and GAD activity in gastric cancer. Med. Sci. Monit. 7, 377-381.

Mazurkiewicz M., Opolski A., Wietrzyk J., Radzikowski C. and Kleinrok Z. (1999). GABA level and GAD activity in human and mouse normal and neoplastic mammary gland. J .Exp. Clin. Cancer. Res. 18, 247-253.

McKerman R.M. and Whiting P.J. (1996). Which $GABA_A$-receptor subtypes really occur in the brain? Trends Neurosci. 19, 139-143.

Meier E., Hertz L. and Schousboe A. (1991). Neurotransmitters as developmental signals. Neurochem. Int. 19, 1-15.

Minuk G.Y. and Gauthier T. (1993). The effect of γ-aminobutyric acid on hepatic regenerative activity following partial hepatectomy in rats. Gastroenterology 104, 217-221.

Minuk G.Y., Kren B.T., Xu R., Zhang X.K., Burczynski F.J., Mulrooney N.P., Fan G. and Steer C.J. (1997). The effect of changes in hepatocyte membrane potential on immediate-early protooncogene expression following partial hepatectomy in rats. Hepatology 25, 1123-1127.

Nilssen L.S., Odegard J., Thoresen G.H., Molven A., Sandnes D. and Christoffersen T. (2004). G protein-coupled receptor agonist-stimulated expression of ATF3/LRF-1 and c-myc and comitogenic effects in hepatocytes do not require EGF receptor transactivation. J. Cell. Physiol. 201, 349-358.

Opolski A., Mazurkiewicz M., Wietrzyk J., Kleinrok Z. and Radzikowski C. (2000). The role of GABA-ergic system in human mammary gland pathology and in growth of transplantable murine mammary cancer. J. Exp. Clin. Cancer Res. 19, 383-390.

Owens D.F. and Kriegstein A.R. (2002). Is there more to GABA than synaptic inhibition? Nat. Rev. Neurosci. 3, 715-727.

Palanivelu R., Brass L., Edlund A.F. and Preuss D. (2003). Pollen tube growth and guidance is regulated by POP2, an Arabidopsis gene that controls GABA levels. Cell 114, 47-59.

Prenzel N., Zwick E., Daub H., Leserer M., Abraham R., Wallasch C. and Ullrich A. (1999). EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. Nature 402, 884-888.

Roberts S.S., Mori M., Pattee P., Lapidus J., Mathews R., O'Malley J.P., Hsieh Y.C., Turner MA., Wang Z., Tian Q., Rodland M.J., Reynolds C.P., Seeger RC. and Nagalla S.R. (2004). GABAergic system gene expression predicts clinical outcome in patients with neuroblastoma. J. Clin. Oncol. 22, 4127-4134.

Rosen L.B., Ginty D.D., Weber M.J. and Greenberg M.E. (1994). Membrane depolarization and calcium influx stimulate MEK and MAP kinase via activation of Ras. Neuron 12, 1207-1221.

Steward F.C., Thompson J.F. and Dent C.E. (1949). γ-aminobutyric acid: a constituent of the potato tuber? Science 110, 439-440.

Takayama C. and Inoue Y. (2004). GABAergic signaling in the developing cerebellum. Anat. Sci. Int. 79, 124-136.

Tamayama T., Kanbara K., Maemura K., Kuno M. and Watanabe M. (2001). Localization of GABA, GAD65, GAD67 in rat epiphyseal growth plate chondrocytes. Acta. Histochem. Cytochem. 34, 201-206.

Tamayama T., Maemura K., Kanbara K., Hayasaki H., Yabumoto Y., Yuasa M. and Watanabe M. (2005). Expression of $GABA_A$ and $GABA_B$ receptors in rat growth plate chondrocytes: Activation of the GABA receptors promotes proliferation of mouse chondrogenic ATDC5 cells. Mol. Cell. Biochem. 273, 117-126.

Tatsuta M., Iishi H., Baba M., Nakaizumi A., Ichii M. and Taniguchi H. (1990). Inhibition by gamma-amino-n-butyric acid and baclofen of gastric carcinogenesis induced by N-methyl-N'-nitro-N-nitrosoguanidine in Wistar rats. Cancer Res. 50, 4931-4934.

Tatsuta M., Iishi H., Baba M., Nakaizumi A., Uehara H. and Taniguchi H. (1992). Effect of gamma-butyrolactone on baclofen inhibition of gastric carcinogenesis induced by N-methyl-N'-nitro-N-nitrosoguanidine in Wistar rats. Oncology 49, 123-126.

Ullrich A. and Schlessinger J. (1990). Signal transduction by receptors with tyrosine kinase activity. Cell 61, 203-212.

Waagepetersen H.S., Sonnewald U. and Schousboe A. (1999). The GABA paradox: Multiple roles as metabolite, neurotransmitter, and neurodifferentiative agent. J. Neurochem. 73, 1335-1342.

Wang F.Y., Maemura K., Hirata I., Katsu K. and Watanabe M. (2000). Immunohistochemical study on GABAergic system in rat and human large intestine. Bull. Osaka Med. 46, 25-34.

Wang F.Y., Watanabe M., Zhu R.M. and Maemura K. (2004). Characteristic expression of γ-aminobutyric acid and glutamate decarboxylase in rat jejunum and its relation to differentiation of epithelial cells. World J. Gastroenterol 10, 3608-3611.

Wang J.M., Johnston P.B., Ball B.G. and Brinton R.D. (2005). The neurosteroid allopregnanolone promotes proliferation of rodent and human neural progenitor cells and regulates cell-cycle gene and protein expression. J. Neurosci. 25, 4706-4718.

Watanabe M., Maemura K., Kanbara K., Tamayama T. and Hayasaki H. (2002). GABA and GABA receptors in the central nervous system and other organs. Int. Rev. Cytol. 213, 1-47.

Wolff J.R., Joó F. and Dames W. (1978). Plasticity in dendrites shown by continuous GABA administration in superior cervical ganglion of adult rat. Nature 274, 72-74.

Yan Y., Shirakabe K. and Werb Z. (2002). The metalloprotease Kuzubanian (ADAM10) mediates the transactivation of EGF receptor by G protein-coupled receptors. J. Cell Biol. 158, 221-226.

Yang Z. (2003). GABA, a new player in the plant mating game. Dev. Cell 5, 185-186.

Yarden Y. (2001). The EGFR family and its ligands in human cancer: signaling mechanisms and therapeutic opportunities. Eur. J. Cancer 37, S3-S8.

Zhang M., Gong Y., Assy N. and Minuk Y. (2000). Increased GABAergic activity inhibits a-fetoprotein mRNA expression and the proliferation activity of the HepG2 human hepatocellular carcinoma cell line. J. Hepatol. 32, 85-91.

Zhang M., Song G. and Minuc GY. (1996). Effects of hepatic stimulator substance, herbal medicine selenium/vitamin E, and ciprofloxacin on cirrhosis in the rat. Gastroenterology 110, 1150-1155.

Zwick E., Hackel P.O., Prenzel N. and Ullrich A. (1999). The EGF receptor as central transducer of heterologous signaling systems. Trends Pharmacol. Sci. 20, 408-412.

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