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Exciting Times beyond the Brain: Metabotropic Glutamate Receptors in Peripheral and Non-Neural Tissues

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***Abstract*——Metabotropic glutamate (mGlu) receptors are G-protein-coupled receptors expressed primarily on neurons and glial cells, where they are located in the proximity of the synaptic cleft. In the central nervous system (CNS), mGlu receptors modulate the effects of L-glutamate neurotransmission in addition to that of a variety of other neurotransmitters. However, mGlu receptors also have a widespread distribution outside the CNS that has been somewhat neglected to date. Based on this expression, diverse roles of mGlu receptors have been suggested in a variety of processes in health and disease including controlling hormone production in the adrenal gland and pancreas, regulating mineralization in the developing cartilage, modulating lymphocyte cytokine production, directing the state of differentiation in embryonic stem cells, and modulating gastrointestinal secretory function. Understanding the role of mGlu receptors in the periphery will also provide a better insight into potential side effects of drugs currently being developed for neurological and psychiatric conditions. This review summarizes the new potential roles of mGlu receptors and raises the possibility of novel pharmacological targets for various disorders.**

I. Introduction

L-Glutamate is the major excitatory amino acid neurotransmitter in the mammalian central nervous system (CNS[1]) (Robinson and Coyle, 1987; Conn, 2003). It signals through two types of receptors, ionotropic glutamate (iGlu) and metabotropic glutamate (mGlu) receptors. iGlu receptors act as glutamate-gated ion channels and regulate rapid responses upon activation, whereas mGlu receptors are G-protein-coupled receptors modulating signal transduction cascades (Cryan and Dev, 2008). On the basis of their intracellular signal transduction mechanisms, agonist pharmacology, and sequence homologies, mGlu receptors have been further divided into three subfamilies, termed group I (mGlu1 and mGlu5), group II (mGlu2 and mGlu3), and group III (mGlu4, mGlu6, mGlu7, and mGlu8) (Conn, 2003; Niswender and Conn, 2010). In the CNS, activation of mGlu receptors from group I stimulate phosphoinositide hydrolysis with subsequent formation of inositol 1,4,5-triphosphate and diacylglycerol, whereas receptors from group II and III induce a decrease on the intracellular levels of cAMP upon activation (Conn, 2003; Niswender and Conn, 2010; O'Connor and Cryan, 2010) (see Fig. 1).

In the CNS, mGlu receptors are expressed by neurons and glia, where they locate in the proximity of the synaptic cleft. Therefore, mGlu receptors can modulate not only the effect of glutamate in the postsynaptic neurons

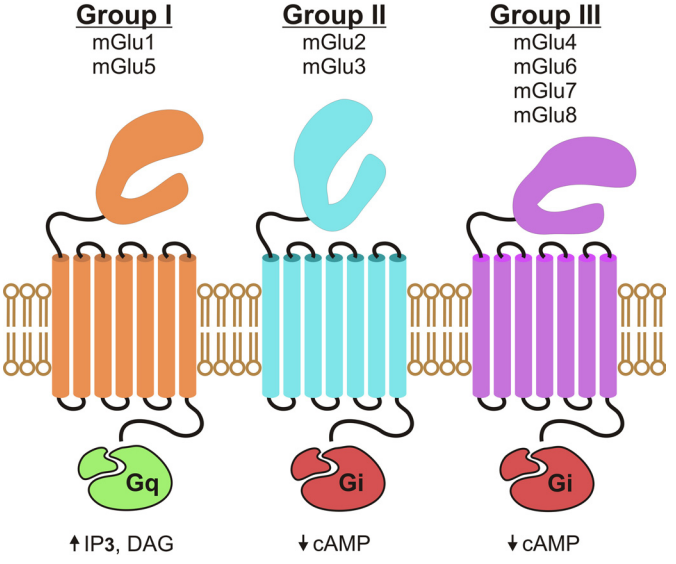

FIG. 1. mGlu receptor families. mGlu receptors are classified into three families: group I, group II, and group III. In the CNS, activation of mGlu receptors from group I induces phosphoinositide hydrolysis with formation of inositol 1,4,5-triphosphate and diacylglycerol, whereas activation of receptors from groups II and III induce a decrease on the intracellular levels of cAMP (Conn, 2003; Niswender and Conn, 2010; O'Connor and Cryan, 2010).

[1]Abbreviations: 4C3HPG, (*S*)-4-carboxy-3-hydroxyphenylglycine; 5-FU, 5-fluorouracil; ACPD, 1-aminocyclopentane-*cis*-1,3-dicarboxylic acid; AIDA, (*R*,*S*)-1-aminoindan-1,5-dicarboxylic acid (UPF523); AMN082, *N*,*N*′-bis(diphenylmethyl)-1,2-ethanediamine; APDC, (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylate; BAY36-7620, (3a*S*,6a*S*)-hexahydro-5-methylene-6a-(2-naphthalenylmethyl)-1*H*-cyclopenta[c]furan-1-one; BBB, blood-brain barrier ; BMVEC, brain microvascular endothelial cell; CNS, central nervous system; CPPG, (*R*,*S*)-α-cyclopropyl-4-phosphonophenylglycine; CSNB, congenital stationary night blindness; DCG-IV, (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine; DHPG, (*S*)-3,5-dihydroxyphenylglycine; EAE, experimental autoimmune encephalomyelitis; EB, embryoid body; EPSP, excitatory postsynaptic potential; ERK, extracellular signal-regulated kinase; ES, embryonic stem; GERD, gastroesophageal reflux disease; GI, gastrointestinal ; IBS, irritable bowel syndrome; iGlu, ionotropic glutamate; IL, interleukin; L-AP4, L-(+)-2-amino-4-phosphonobutyric acid; LPS, lipopolysaccharide ; LY341495, (2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid; LY367385, (*S*)-(+)-α-amino-4-carboxy-2-methylbenzeneacetic acid; MAP4, (*S*)-2-amino-2-methyl-4-phosphonobutanoic acid; mGlu, metabotropic glutamate; MPEP, 2-methyl-6-(phenylethynyl)pyridine; MSG, monosodium glutamate; NMDA, *N*-methyl-D-aspartic acid; NO, nitric oxide; PHCCC, *N*-phenyl-7-(hydroxyimino)cyclopropa[*b*]chromen-1*a*-carboxamide; PLC, phospholipase C; PMN, polymorphonuclear leukocyte; ROS, reactive oxygen species; RT-PCR, reverse transcription-polymerase chain reaction; SNL, spinal nerve lesion; Th-17, IL-17-producing T helper; TLESR, transient lower esophageal sphincter relaxation.

and accessory cells but also the release of glutamate and other neurotransmitters in presynaptic and heterosynaptic localizations (Schoepp, 2001; O'Connor and Cryan, 2010). This has led to an increased drug discovery effort in developing selective mGlu receptor ligands for a variety of CNS conditions ranging from Parkinson's disease and schizophrenia to stress-related disorders (Conn, 2003; Swanson et al., 2005).

In addition, growing evidence indicates that mGlu receptors are expressed in the periphery. Whereas some of those tissues, such as the heart (Gill et al., 2007) and the adrenal glands (Hinoi et al., 2004) are known to receive direct glutamatergic innervation, mGlu receptors are also present in cells that are not under such synaptic control, such as lymphocytes (Pacheco et al., 2006). The fact that mGlu receptors have roles other than regulation of synaptic transmission intensifies discovery efforts into exploiting pharmacology originally directed to the CNS toward multiple new targets in the periphery. In the last few years, this field has further benefited from the appearance of new selective compounds providing better tools to examine not only the classic pathways associated with glutamatergic signaling but also the role of mGlu receptors in non-neural tissues. Moreover, an understanding of the pharmacology of glutamate in peripheral tissues will be vital for predicting potential adverse effects of CNS-targeted mGlu receptor-based therapies.

II. Sources of Peripheral Glutamate in Health and Disease

To understand the roles of mGlu receptors in the periphery, it is important to have an appreciation of where their ligand L-glutamate is found (see Fig. 2). L-Glutamate is a naturally occurring amino acid in the mammalian body. Although L-glutamate comprises 4 to 15% of all amino acids in natural proteins, plasma glutamate concentrations are rather low, ranging between 20 and 50 μM in healthy persons (Graham et al., 2000; Vaccaro et al., 2007). In contrast, the levels of L-glutamate in whole brain can locally reach 10^4 μM, although the concentrations in the extracellular fluid are very low, normally reaching less than 2 μM. In the CNS, L-glutamate must be locally synthesized, because it does not cross the blood-brain barrier (BBB) (Hawkins, 2009).

During the digestion process, L-glutamate is released to the lumen of the small intestine when dietary proteins are cleaved. In addition, some food products contain significant amounts of free L-glutamate (i.e., not bound to a polypeptide). The major source of dietary free L-glutamate is the salt monosodium glutamate (MSG), which is used as a

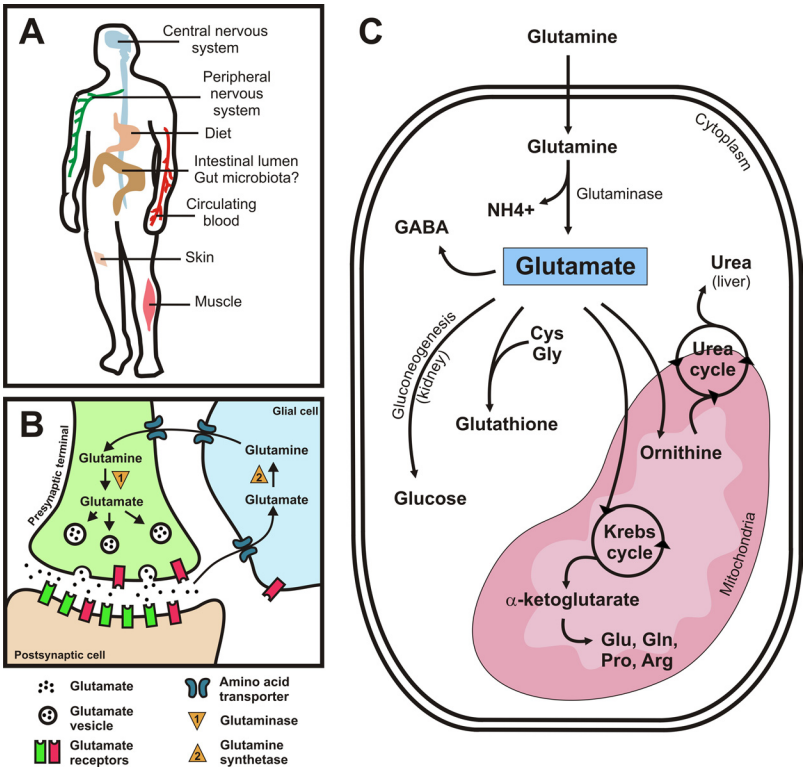

FIG. 2. L-Glutamate sources and metabolism. A, physiological sources of glutamate. L-Glutamate is locally synthesized in the CNS; peripheral nervous tissues also contain L-glutamate, which can eventually reach cells and tissues in the proximity of nerve terminals. L-Glutamate is also found in the diet (and therefore in the intestinal contents), in plasma, skeletal muscle, and skin. B, glutamate synthesis and cycling between neurons and glial cells. L-Glutamate is synthesized from L-glutamine by presynaptic neurons, stored, and released upon stimulation into the synaptic cleft, where it reaches target cells. Excess extracellular L-glutamate can be converted into the less toxic amino acid L-glutamine by glia, which is then transported into the presynaptic cell. C, metabolic fates of glutamate. L-Glutamate is a precursor for the neurotransmitter GABA and the antioxidant molecule glutathione, and it is also involved in various biochemical pathways relevant for energy production and nitrogen metabolism.

flavor-enhancing food additive. Both MSG and L-glutamate derived from cleaved proteins are able to target epithelial cells in the luminal surface of the gastrointestinal (GI) tract (Blachier et al., 2009). Postabsorptive levels of plasma L-glutamate increase when dietary protein intake is poor, a response attributed to changes in de novo production (Matthews and Campbell, 1992). In humans, ingestion of small doses of glutamate results in first-pass metabolism oxidation by the splanchnic bed. However, intake of larger doses of MSG (150 mg/kg) is followed by an increase in plasma L-glutamate concentrations and a more moderate rise in muscle glutamate concentrations for 1 to 2 h (Graham et al., 2000).

In the periphery, other potential sites for endogenous synthesis of L-glutamate include the peripheral nervous tissues and more importantly the autonomic ganglia (see Fig. 2) (Gill and Pulido, 2005). Cells and tissues in close proximity to nerve terminals can therefore be affected by L-glutamate release. Immunohistochemistry studies have shown that normal human skin, for instance, stains positive for glutamate; interestingly, in addition to the cells with a known nociceptive function, macrophage-like cells in the dermis and epidermis also contain L-glutamate (Nordlind et al., 1993). This immunoreactivity is further elevated in the inflamed skin (Nordlind et al., 1993). During inflammation, the increase in endothelial permeability facilitates L-glutamate passage from plasma to the inflamed region (Carlton, 2005). In some cases, inflamed skin can lead to pruritus (itching); interestingly, the patent literature suggests that mGlu5 receptor antagonists may be used to treat or prevent pruritus associated with dry skin and parasite infection (Gasparini et al., 2002).

Other disorders involving inflammation are also associated with L-glutamate extravasation toward the affected region: in models of acute lung injury such as heat-induced lung inflammation and hyperoxia-induced neonatal lung inflammation, tissue damage is accompanied by the release of high levels of glutamate (Wang et al., 2009; Yang et al., 2009). Likewise, synovial fluid isolated from the knee joints in patients suffering from rheumatoid arthritis and animal models of arthritis contain increased L-glutamate levels (Flood et al., 2007). For human disorders associated with altered peripheral L-glutamate levels, please refer to Table 1.

In some cases, a pathological condition can be associated with higher plasma L-glutamate levels thus allowing for peripheral mGlu stimulation and potential side effects. This is the case for patients with cancer (Dröge et al., 1988). It is believed that the metabolic properties of tumors combined with an altered metabolism in patients with cancer contribute to the abnormal L-glutamate plasma concentrations in these patients (Dröge et al., 1988). Elevated plasma concentrations of L-glutamate are also observed in patients infected with HIV (Ferrarese et al., 2001). In conditions such as migraine, in addition to high levels of glutamate in the plasma, there is an increased glutamate uptake in platelets that is believed to be a compensatory response (Vaccaro et al., 2007). Increased L-glutamate uptake by other tissues would also be possible under such conditions (see Fig. 2); additional compensatory measures could involve modulation of expression of mGlu receptors or their signaling machinery, extending the autoreceptor-like function attributed to mGlu receptors in the CNS to prevention of L-glutamate excitotoxicity in the periphery. Although the relevance of these increased glutamate concentrations under pathological conditions to disease manifestation and natural history is not always clear, it points to an important area for future research.

III. Evolutionary Aspects of Metabotropic Glutamate Receptors

L-Glutamate's function as a signaling molecule appeared early in evolution; this is supported by the fact that glutamate receptor-like genes have been identified in plants and multiple lower animals, mostly with similarity to ionotropic glutamate receptors of mammals in terms of both primary structure and domain organization (Chiu et al., 1999; Tikhonov and Magazanik, 2009). It is noteworthy that mGlu receptor orthologs have also been described in several species, including *Drosophila melanogaster* (Parmentier et al., 1996; Mitri et al., 2004), *Caenorhabditis elegans* (Pin and Acher, 2002),

TABLE 1
Disorders associated with altered peripheral L-glutamate levels

Pathological Condition	L-Glutamate Source	References
Increased L-glutamate levels		
Acute and chronic myeloid leukemia	Plasma	Singh et al., 1989
Arthritis	Synovial fluid	Flood et al., 2007; McNearney et al., 2000
Cancer	Plasma	Dröge et al., 1987, 1988
Glaucoma	Vitreous body	Dreyer et al., 1996
Immune deficiency-HIV	Plasma	Ferrarese et al., 2001
Inflamed skin	Skin	Nordlind et al., 1993
Migraine	Plasma	Vaccaro et al., 2007
Decreased L-glutamate levels		
Emphysema	Skeletal muscle	Engelen et al., 2000
Periodontitis	Gingival fluid	Téllez et al., 2008
Sepsis with acute liver dysfunction	Plasma	Poeze et al., 2008

and *Dictyostelium discoideum* (amoeba) (Taniura et al., 2006).

The primary structure as well as the pharmacology of mGlu receptors seems to be evolutionarily well conserved among *D. melanogaster*, *C. elegans*, and higher mammals. Sequence analysis of the *C. elegans* genome showed the presence of a homolog for each of the vertebrate mGlu receptor groups, which strongly suggests that the three families of mGlu receptors (Fig. 1) were already present in the common ancestor of nematodes and vertebrates (Pin and Acher, 2002). In addition, three-dimensional-model analysis between *D. melanogaster* mGlu receptor orthologs and the mammalian group II mGlu receptors indicates that there has been a strong selection pressure during evolution to maintain receptor domains and amino acid identity responsible for the ligand recognition and selectivity of mGlu receptors (Parmentier et al., 2000).

In *D. melanogaster*, two kinds of mGlu receptors have been reported: DmGluRA and DmXR. DmGluRA is of special interest as it is expressed at the glutamatergic neuromuscular junction and shares a very similar pharmacological profile with its mammalian orthologs, mGlu2 and mGlu3 (Parmentier et al., 1996; Mitri et al., 2004). On the other hand, in the social amoeba *D. discoideum*, only one genomic mGlu receptor homolog, DdmGluPR, has been identified, and it seems to have a role in the signaling cascade of aggregate formation upon starvation and chemotaxis toward cAMP (Taniura et al., 2006).

The *D. discoideum* genome contains just one mGlu-like receptor, *C. elegans* has three, and higher mammals possess eight variants. This and the evolutionary conservation of the three pharmacological groups of mGlu receptors from *C. elegans* to human strongly suggest that mGlu subtypes emerged via a series of gene duplications throughout evolution. The above evidence further indicates that along early evolutionary stages, mGlu receptors may have developed to command glutamate signaling in multiple contexts forming the mGlu receptor system as key modulator of both neural and non-neural events in mammalian physiology.

IV. Gastrointestinal Tract: a Multitasking Site for Metabotropic Glutamate Receptors

There is a strong body of evidence supporting the role of glutamate as a primary neurotransmitter in the vagal circuitry commanding key GI functions (for review, see Hornby, 2001). mGlu receptors seem to be relevant not only for modulation of GI vagovagal reflexes (Young et al., 2007, 2008) but also for the process of digestion as a whole. Here we describe these events individually and sequentially, and a summary of mGlu receptor expression in GI and other peripheral tissues is presented in Table 2.

A. The Initiation Sequelae of a Meal

In the mucosa of the upper digestive tract, mGlu receptors together with taste receptor type 1 (T1R) are believed to act as chemosensory receptors responsible for the *umami* basic taste (savoriness attributable to the carboxylate anion of MSG) thus contributing to the early

TABLE 2
Localization of mGlu receptors in peripheral tissues

This table does not include sensory tissues.

Tissue	Group I		Group II		Group III			
	mGlu1	mGlu5	mGlu2	mGlu3	mGlu4	mGlu6	mGlu7	mGlu8
Adrenal gland	√	√	√	√	√		√	
Bone cells	√				√	√		√
Cardiac muscle	√	√	√	√				
Cartilage	√		√		√			√
Colon		√			√		√	√
Dendritic cells					√			
Duodenum	√				√			√
Endothelium	√	√			√			
Esophagus	√				√			
Gall bladder					√			
Ileum		√	√	√				√
Kidney			√	√	√			
Larynx					√			
Liver		√		√				
Lung				√	√			
Mammary gland					√			
Ovary			√	√				
Pancreas		√	√	√	√			√
Salivary gland					√			
Skin	√	√	√	√				
Stem cells		√			√			
Stomach	√	√	√	√	√	√	√	√
Synovial cells					√			√
T cells	√	√						
Testis	√	√	√	√	√	√		√
Thymus	√	√	√	√	√			
Tumors/transformed cells	√	√		√	√			
Uterine cervix					√			

detection of proteinaceous content in a meal (for reviews, see Shigemura et al., 2009; Yasumatsu et al., 2009). mGlu1 and mGlu4 receptor variants have been described in the rat taste buds by several techniques, including RT-PCR, RNase protection assays, and in situ hybridization (Chaudhari et al., 1996, 2000; San Gabriel et al., 2005). It is noteworthy that these truncated forms of mGlu receptors, also known as taste mGlu1 and taste mGlu4, lack a great portion of the glutamate-binding domain, (Chaudhari et al., 2009); consequently, the effective concentration of L-glutamate required to activate the taste mGlu receptor variants is higher than that needed for the full-length receptors (Chaudhari et al., 2000, 2009).

In support of a role for mGlu4 in taste, the group III mGlu receptor antagonist CPPG decreased the amount of aversion for MSG and other amino acids when MSG served as the conditioned stimulus in a taste aversion test (Eschle et al., 2009). However, when CPPG was mixed with these amino acids, the strength of the learned taste aversions and cross-generalization were either decreased or increased, indicating that the antagonist was not only able to reduce the intensity of the stimulus experience but also changed the qualities of the sensory experience (Eschle et al., 2009). These authors propose that multiple receptors are involved in amino acid taste and that taste mGlu4 receptors contribute to the taste of MSG and at least some other L-amino acids.

Other mGlu receptors have also been identified in taste sensing tissues, including mGlu2 and mGlu3 (Toyono et al., 2007). In addition, focal expression of mGlu4 was identified by immunohistochemistry in healthy human salivary glands (Chang et al., 2005), a finding that has not yet been supported by functional characterization.

B. Esophagus and Stomach

The lower esophageal sphincter plays a key role in preventing gastric reflux. The main cause of gastroesophageal reflux disease (GERD) is transient lower esophageal sphincter relaxation (TLESR) (Blackshaw, 2008). L-Glutamate is an important activator of vagal pathways mediating TLESR (Partosoedarso and Blackshaw, 2000); furthermore, retrograde tracing studies indicate that mGlu1–8 receptor proteins are expressed in these gastric vagal afferents (Page et al., 2005). Therefore, it was hypothesized that TLESR may be associated with impairment in mGlu receptor signaling. In a conscious ferret model of TLESR induced by a high gastric load, the prototypical mGlu5 receptor antagonist MPEP prevented sphincter relaxation dose-dependently, reaching a maximal inhibition of 71%. MPEP also reduced reflux episodes and increased basal lower esophageal sphincter pressure (Frisby et al., 2005). Similar results were obtained by administration of MPEP in a dog model of TLESR (Jensen et al., 2005). In the ferret, stronger inhibition of TLESR was achieved by a different mGlu5 receptor selective antagonist, 3-((2-methyl-1,3-thiazol-4-yl)ethynyl)pyridine; on the other hand, the group I receptor agonist DHPG tended to increase TLESR. The group II receptor agonist APDC had no effect, whereas the group III receptor agonist L-AP4 slightly reduced TLESR. It is noteworthy that the mGlu8 receptor agonist (*S*)-3,4-dicarboxyphenylglycine was able to inhibit TLESR by 54% (Frisby et al., 2005). The promising therapeutic potential of mGlu5 receptor antagonists for patients with gastroesophageal reflux disease has been further exploited in clinical trials: the mGlu5 negative allosteric modulator ADX10059 (Bolea et al., 2004) improved symptoms in patients with GERD who reported fewer and shorter reflux episodes than those treated with placebo (Keywood et al., 2009); however, ADX10059 administration was associated with central side effects such as dizziness, which was attributed to the compound's rapid absorption. Better tolerability was achieved by a modified release formulation (Zerbib et al., 2010).

mGlu1 receptor is expressed in rat esophagus mucosa, and mGlu4 is present in both mucosa and muscle layer (Akiba et al., 2009); likewise, normal human esophagus also presents positive immunoreactivity for mGlu4 (Chang et al., 2005). However, the potential effects of directly targeting these receptors in the esophagus mucosa or muscle are unknown to date. It is noteworthy that group I mGlu receptors seem to also have a role in gastroesophageal pain sensitivity, in that the mGlu5 receptor antagonist 3-((2-methyl-1,3-thiazol-4-yl)ethynyl)pyridine dose-dependently inhibited mechanosensitivity in vitro (Slattery et al., 2006). These data suggest an interesting additional potential for the use of mGlu5-directed compounds for treatment of patients with GERD, who may show increased esophageal perception as a result of chronic tissue damage.

As for the stomach, various degrees of mRNA expression have been described for mGlu1–8 in the different cell components of the rat stomach mucosa (Nakamura et al., 2010). In the case of mGlu receptor protein levels, intense mGlu2/3 staining was found in both parietal and endocrine cells, suggesting a role in the regulation of gastric acid and gastrin secretion (Gill and Pulido, 2001). mGlu1, on the other hand, is present in the apical membrane of chief cells, which suggests that it may be involved in the expression of pepsinogen in the stomach mucosa (San Gabriel et al., 2007). In general, mGlu receptor expression in the stomach mucosa is believed to contribute to sensing levels of luminal L-glutamate, although functional evidence supporting this hypothesis is not available to date.

C. Duodenal Protection

Akiba et al. (2009) studied the effect of L-glutamate in the defense mechanisms of the rat duodenal mucosa. Perfusion of the duodenum with glutamate increased intracellular pH and mucus gel thickness in vivo and

decreased acid-induced epithelial cell injury in vivo (Akiba et al., 2009). This is indicative of a protective role for luminal L-glutamate in addition to the physiological role of early detection of a meal. They also showed that mGlu1 and mGlu4 were expressed in the mucosa of both gastric antrum and duodenum. It is noteworthy that the group III mGlu receptor agonist L-AP4 mimicked duodenum-protecting effects produced by L-glutamate; in contrast, the mGlu1/5 receptor agonist DHPG increased intracellular pH but had no effect on mucus gel thickness. Simultaneous administration of the group III mGlu receptor antagonist MAP4 with L-glutamate prevented glutamate-induced alkalinization and mucus secretion, whereas the mGlu1/5 receptor antagonist AIDA partially inhibited alkalinization but had no effect on mucus secretion induced by L-glutamate (Akiba et al., 2009). This recent evidence suggests that patients suffering from duodenal ulcers may benefit from treatments directed against mGlu4 or mGlu1/5 signaling.

D. Regulation of Intestinal Fluid Secretion

Immunohistochemistry studies have demonstrated the presence of mGlu2/3 in submucosal neurons of rat jejunum and ileum (Larzabal et al., 1999) and of mGlu1α and mGlu5 in submucosal neurons of the guinea pig ileum. These neurons have a secretomotor function, which means they regulate the process of absorption and secretion of fluid from the faeces and toward the lumen, respectively (Hu et al., 1999; Liu and Kirchgessner, 2000). The group I mGlu receptor agonist DHPG can induce mGlu5 internalization in isolated enteric neurons, an effect mimicked in ex vivo guinea pig ileum preparations by stimulating villi movement with gaseous bubbling, thus indicating that mechanical mucosal stimulation may be an important mechanism for activation of enteric mGlu5 receptors in vivo (Liu and Kirchgessner, 2000). In submucosal neurons, glutamate and group I mGlu receptor agonists evoke slow depolarizations, an effect that is sensitive to the group I mGlu receptor antagonist (*S*)-4-carboxyphenylglycine (Hu et al., 1999; Ren et al., 2000). On the other hand, slow excitatory postsynaptic potentials (EPSPs) were not affected by the antagonist (Ren et al., 2000). L-Glutamate acting via group I mGlu receptors has been shown to suppress slow EPSPs and potentiate slow inhibitory postsynaptic potentials in submucosal neurons (Ren et al., 1999). However, conflicting evidence also shows that the mGlu5 receptor antagonist MPEP, as well as (*S*)-4-carboxyphenylglycine, is able to depress slow EPSPs in submucosal neurons (Liu and Kirchgessner, 2000). It is noteworthy that another piece of work shows that expression of functional mGlu5 receptors in ileum and colon of guinea pig, rat, and mouse is restricted to enteric glia (Nasser et al., 2007). Glial c-fos immunoreactivity and phosphorylated ERK1/2 levels are increased upon mGlu5 activation in guinea pigs, and chemically induced colitis was associated with redistribution of the receptor. On the other hand, a mouse model of colitis (the IL-10 gene-deficient mouse, characterized by spontaneous chronic intestinal inflammation) shows significantly lower levels of glial mGlu5 receptor expression in colon myenteric plexus (Nasser et al., 2007).

The luminal environment in the large intestine is markedly different to that of the small intestine, and one characteristic of colonic epithelium is that there is little or no transfer of amino acids from the colonic lumen to portal blood except for a short period after birth. As such, amino acids including L-glutamate must be taken into colonocytes from arterial blood (Blachier et al., 2009). However, mGlu receptors can be found in colon mucosa, suggesting that L-glutamate plays a role in colon mucosal physiology. mGlu4 receptor has been detected by immunohistochemistry in normal human colon epithelium (Chang et al., 2005). Recently, we have shown that the mouse colon mucosa also expresses both mRNA and protein for mGlu7 (Julio-Pieper et al., 2010). Moreover, we demonstrate that the selective mGlu7 receptor agonist *N*,*N*′-bis(diphenylmethyl)-1,2-ethanediamine (AMN082) induces an increase in fecal water content in a stress-induced defecation paradigm. Furthermore, the mGlu7 receptor agonist can act directly on the colon, as shown by ex vivo experiments in which pretreatment of colon tissue with AMN082 induces a higher secretory response to the muscarinic receptor agonist bethanechol (Julio-Pieper et al., 2010). This effect is dependent on the presence of submucosal neurons, because when tissues were treated with a nerve toxin, the secretory response was markedly blunted (Julio-Pieper et al., 2010). Together, these data indicate that glutamatergic activation of colonic mGlu7 receptor could be a component in the pathophysiology of secretory disorders such as diarrhea and a significant factor underlying stress-induced diarrhea. Thus it may play a role in functional intestinal disorders such as irritable bowel syndrome (IBS) that are comorbid with chronic stress states (Quigley, 2006; Clarke et al., 2009; Gros et al., 2009). Stressful life events and experimental stress also induce exacerbation of symptoms and visceral hypersensitivity in patients with functional GI disorders (Whitehead, 1992; Posserud et al., 2004). The foregoing evidence supports the idea of a complex interaction between the GI tract and the brain, namely the brain-gut axis. Thus mGlu7 may not only regulate the central components of physiological stress (Cryan et al., 2003; Mitsukawa et al., 2006) but may also have a role to play peripherally in the regulation of fluid and electrolyte transport, which is significantly disrupted in diseases such as IBS, by enhancing colonic secretory activity.

E. Intestinal Motility

Myenteric neurons in the enteric nervous system are a major site of expression of mGlu receptors in the gut. mGlu2/3 has been found by immunohistochemistry in myenteric neurons of rat jejunum and ileum (Larzabal et al., 1999). In addition, mouse colon homogenates con-

taining the muscle layers together with the enteric nerves contain mGlu7 protein, although the administration of an mGlu7 receptor agonist did not modify fecal pellet output upon stress (Julio-Pieper et al., 2010). RT-PCR showed weak expression of mGlu4 and -6, and a strong expression of mGlu7 and -8 in longitudinal muscle with adherent myenteric plexus obtained from rat duodenum and ileum. The presence of mGlu8 receptor in the enteric nervous system of human, rat, and guinea pig has also been shown by immunohistochemistry (Tong and Kirchgessner, 2003). An accelerating effect on guinea pig colon motility and longitudinal muscle contractions was observed after the application of mGlu8 receptor agonists on the isolated tissue. On the other hand, mGlu8 receptor antagonists used alone were able to decrease motility (Tong and Kirchgessner, 2003), which indicates a probable tonic effect of mGlu8 on colonic contractions.

F. Microbiota Composition

The composition of intestinal flora is considered an important factor for both the maintenance of good health and development of disease states such as inflammatory bowel disease and IBS (Clarke et al., 2009). In the specific case of IBS, patients report recurrent abdominal pain in combination with either diarrhea or constipation, which may arise due to an impairment on intestinal motility (Clarke et al., 2009). The amount of various fecal bacterial strains is different in patients with IBS compared with healthy control subjects (Kassinen et al., 2007; Kerckhoffs et al., 2009); in addition, the composition of their intestinal microflora seems to change more over time (Mättö et al., 2005). Moreover, alterations in the fecal microbiota have also been found in an early life-stress model of IBS (O'Mahony et al., 2009). Gut bacteria contain a relatively high internal pool of glutamate that responds by increasing as the external environment becomes hyperosmotic (Yan, 2007). It is plausible that L-glutamate of bacterial origin has the potential to activate mGlu receptors in the GI tract. Thus, further studies must investigate whether the ability of different bacteria to accumulate/release glutamate plays a role in normal gut function and in GI disorders such as IBS and inflammatory bowel disease. Furthermore, the existence of multiple nodes of communication between the brain and gut is supported by increasing evidence from germ-free animals, probiotic and antibiotic administration, and microflora transplantation studies, which suggest that the enteric microbiota can directly influence brain-gut axis function (Rhee et al., 2009). This results in perturbations of the stress response (Forsythe et al.,; Verdu, 2009), immune system (Sudo et al., 2002), endocrine system (Turnbaugh and Gordon, 2009), and pain processing (Rousseaux et al., 2007; O'Mahony et al., 2010) and in behavioral changes (Forsythe et al., 2010). Understanding whether L-glutamate from microbiota and thus mGlu receptors play a role in brain-gut axis communication is worthy of further investigation.

V. Metabotropic Glutamate Receptors in Renal and Hepatic Function

Within the liver and kidney, L-glutamate along with L-glutamine plays a critical role in nitrogen metabolism: in the cytosol of hepatocytes, amino groups from most amino acids are transferred to α-ketoglutarate to form L-glutamate, which releases its amino group as ammonia in the mitochondria. In addition, excess ammonia generated in the tissues is combined with glutamate to yield glutamine, the nontoxic alternative for transporting ammonia (Fig. 2) (Watford, 2000). Like the liver, the kidney is an important site for glutamate metabolism. Glutamate is present in high concentrations in renal tubular cells and is a source of ammonia upon deamination (Schoolwerth et al., 1983). Furthermore, glutamate transporters in the kidney are possibly involved in regulating the entry of glutamate to regulate cell volume in response to osmotic stress (Hediger, 1999).

Immunohistochemical analysis showed that group II mGlu receptors were present in various structures within the rat renal cortex. The antibody used in this work binds to both mGlu2 and mGlu3 receptors. Staining was stronger in convoluted proximal tubule than in glomeruli. Strong immunoreactivity of mGlu2/3 was found in the juxtaglomerular apparatus, where a coarse granular, intense perinuclear cytoplasmic staining was observed in the granular cells at the wall of the afferent arteriole (Gill et al., 2000). Although no functional data are reported, the authors suggest that the distribution of mGlu2/3 in the rat kidney may involve a role in regulating water and electrolyte transport; interestingly, a similar role has been described for mGlu7 in the colon epithelium (Julio-Pieper et al., 2010). On the other hand, the presence of mGlu2/3 in the afferent arteriole could be related with the control of renin release (Gill et al., 2000). In the human kidney, focal expression of mGlu4 receptor has been detected in the collecting duct (Chang et al., 2005).

Some of the first data demonstrating the presence of functional mGlu receptors in hepatocytes focused on stimulated inositol monophosphate formation after incubation with the group I receptor agonists quisqualate and ACPD (Sureda et al., 1997). Storto et al. (2000b) confirmed the presence of mGlu5 receptor in hepatocytes by PCR analysis and by immunohistochemistry in neonate and adult rat liver. It is noteworthy that the other member of the group I family, mGlu1, was not detected in rat liver or hepatocytes.

Endogenous L-glutamate, as well as exogenously added L-glutamate, ACPD, and quisqualate, increases the extent of cell damage induced by hypoxia/anoxia in cultured hepatocytes, whereas 4C3HPG, an agonist of mGlu2/3, was inactive. The mGlu5 receptor antagonist

MPEP not only decreased ACPD-associated damage but also was by itself protective against anoxic death (Storto et al., 2000b), indicative of tonically active mGlu5 in hepatocytes. In addition, hepatocytes isolated from mGlu5 knockout mice were less sensitive to hypoxic cell damage than hepatocytes from wild-type mice (Storto et al., 2004). It is noteworthy that mGlu5 also seems to be involved in hepatic failure induced by lipopolysaccharide (LPS), in that MPEP administration markedly reduced the liver damage induced by a previous LPS administration in the mouse (Jesse et al., 2009). Endotoxic shock induced by LPS is characterized by severe hypotension and multisystemic organ failure; all together, this evidence suggests that targeting mGlu5 function may be a new approach for the treatment of liver damage by ischemia-reperfusion, which can develop as a complication of liver transplantation, or during severe episodes of hypotension. Moreover, MPEP also reduced liver necrosis and the production of reactive oxygen species (ROS) induced by acetaminophen in mice (Storto et al., 2003), suggesting that activation of mGlu5 facilitates not only hypoxic but also drug-induced death in hepatocytes.

Liver fibrosis/cirrhosis can be induced in the rat by long-term administration of the organic compound carbon tetrachloride (CCl_4), and it is accompanied by the appearance of mGlu3 receptor immunoreactivity in the macrophages in the fibrous septa starting from the eighth week of treatment (Do et al., 2007). Most of mGlu3 receptor-positive macrophages disappeared in the liver of animals that were left to recover. This result was confirmed by 2-dimensional electrophoresis of whole-liver samples (Do et al., 2007) and indicates that mGlu3 may be involved in the development of inflammatory liver damage.

VI. Metabotropic Glutamate Receptors in the Regulation of Endocrine Function

L-glutamate is an essential component of the neuroendocrine regulation of pituitary hormone secretion. Agents acting at iGlu and mGlu receptors have been shown to modulate the levels of hypothalamic and pituitary factors such as adrenocorticotropin, prolactin, growth hormone, and oxytocin (for reviews see Dhandapani and Brann, 2000; Durand et al., 2008). Moreover, central mGlu receptors seem to modulate corticosterone secretion (Scaccianoce et al., 2003; Mitsukawa et al., 2005, 2006). Less attention has focused on glutamatergic signaling, particularly mGlu receptors, in the target endocrine organs. In this section we will discuss recent data indicating that selective ligands for mGlu subtypes have potential for the treatment of endocrine disorders.

A. Homeostasis of Glucose

The islet of Langerhans, the pancreatic endocrine unit responsible for homeostasis of blood glucose, possesses a glutamatergic system similar to the one found in the CNS (Moriyama and Hayashi, 2003). Under low-glucose conditions, L-glutamate is cosecreted with glucagon from α cells, where it is believed to act in an autocrine fashion (Uehara et al., 2004). Ionotropic receptors are expressed in both α and β pancreatic cells, and their stimulation seems to modulate glucagon/insulin secretion (Uehara et al., 2004). The expression of mRNAs for all eight subtypes of mGlu receptors has been studied by RT-PCR in rat and human pancreatic islets and in rat pancreatic cells. In the study performed by Brice et al. (2002), mRNAs for mGlu receptors 3, 5, and 8 were detected by PCR in all the samples; mGlu2 was found only in β cells, whereas mGlu4 was identified in rat islets. Group I and II mGlu receptor agonists increased the release of insulin in the presence of glucose, whereas a group III mGlu receptor agonist had the opposite effect (Brice et al., 2002).

A further exploration of the role of group I mGlu receptors in pancreatic function showed that endogenous activation of mGlu5 is required for an optimal insulin response to glucose both in clonal β cells and in mice. Confocal analysis showed that mGlu5 was localized mainly at the surface of β cells under basal conditions. Agonist exposure induced a rapid and transient internalization of surface receptors. mGlu5 was also found in purified insulin-containing granules (Storto et al., 2006). In this study, β cells did not respond to mGlu5 receptor agonists that act extracellularly, but a cell-permeant analog of L-glutamate did induce an increase in intracellular Ca^{2+} and insulin secretion. The mGlu5 antagonist MPEP decreased both effects, whereas an antagonist for mGlu1 was inactive. mGlu5 knockout mice had a blunted insulin response after a glucose pulse, an effect that was replicated by the administration of MPEP to wild-type mice. Mice injected with MPEP or lacking the mGlu5 receptor also presented a defective glucagon response after an insulin challenge. This indicates that insulin secretion is under the control of mGlu5 both in clonal β cells and in vivo (Storto et al., 2006).

Another study reports evidence for functional mGlu4, but not other mGlu receptors, in the rat pancreatic islets. The mGlu4 receptor was colocalized with glucagon and pancreatic-polypeptide producing cells, and stimulation of the receptor inhibited secretion of glucagon in a cascade involving inhibition of cAMP production (Uehara et al., 2004). Together with the data provided by Storto et al. (2006), this suggests new potential pharmacological targets for the treatment of endocrine disorders related to glucose metabolism (Tables 4 and 5). In addition, it adds a new range of possible side effects to the emerging mGlu receptor-based therapies for neurological and mood disorders (Lavreysen and Dautzenberg, 2008).

B. Steroidogenic and Reproductive Tissues

The hypothalamus-pituitary-adrenal axis is the most important regulator of the stress response and L-gluta-

mate plays a key role in its activation (Zelena et al., 2005). As for the adrenal gland, no evidence has yet shown a direct action of L-glutamate on glucocorticoid secretion at the adrenal cortex level. However, L-glutamate stimulates the secretion of catecholamines both in the dog adrenal gland and in chromaffin cells isolated from bovine adrenal medulla (Nishikawa et al., 1982; González et al., 1998). These effects are not caused solely by iGlu receptors; they can be mimicked by the group I mGlu agonists ACPD and DHPG (González et al., 1998; Arce et al., 2004). Arce et al. (2004) also report that most of the bovine chromaffin cells isolated from adrenal medulla express group I mGlu receptors, which are at least of two subtypes: mGlu1 and mGlu5a, as demonstrated by immunocytochemistry. mGlu5 receptor mRNA, as well as mGlu7 receptor mRNA, is also present in the mouse and rat adrenal gland (Scaccianoce et al., 2003). A later study also demonstrated the presence of mGlu2/3 and mGlu4a receptors in large-sized type I ganglion neurons and in the small-sized type II adrenal ganglion neurons by immunohistochemical analysis of the rat adrenal medulla (Sarría et al., 2006). However, the functional relevance of this expression has yet to be determined.

In the testis, the presence of glutamate transporters and glutamate decarboxylase enzymes supports the idea that non-neuronal components in this tissue possess their own glutamatergic system (Tillakaratne et al., 1992; Hayashi et al., 2003). Gill et al. (2000) and Gill and Pulido (2001) described an intense and specific signal for mGlu2/3 in the perinuclear cytoplasm of interstitial cells and myoid (contractile) cells in the testis of adult Sprague-Dawley rats. Immunoreactivity with anti-mGlu2/3 is also present in the head of mature spermatids and spermatozoa (Gill et al., 2000; Gill and Pulido, 2001). Contrary to this finding, Storto et al. (2001) reported the presence of mGlu1, -4, and -5, but not mGlu2 or -3 receptor mRNA in the testis of the same strain of rat, a result that was confirmed by Western blot analysis. The mGlu1/5 receptor agonist ACPD induced inositol phospholipid hydrolysis in rat testes preparations, indicating that these receptors are functional (Storto et al., 2001). In testis of the Wistar strain of rat, on the other hand, all mGlu receptors except mGlu7 have been detected by PCR, including an additional splice variant for mGlu2 that was not present in whole rat brain (Takarada et al., 2004). In this study, [^{3}H]glutamate accumulation by testicular fractions was not inhibited by the group I, II, or III mGlu receptor agonists DHPG, DCG-IV, or L-AP4, respectively (Takarada et al., 2004).

Analysis of human testis showed that immunoreactivity for mGlu1a receptor (a splice variant of mGlu1) was restricted to intertubular spaces, whereas mGlu5 receptor was abundantly expressed inside the seminiferous tubuli and in the mid-piece and tail of mature spermatozoa. However, neither the group I agonist quisqualate nor the mGlu5 receptor antagonist MPEP induced changes in human sperm motility (Storto et al., 2001).

Ovarian production of glutathione depends on L-glutamate (see Fig. 2); this process is modulated by gonadotropins and is critical for limiting the damage induced by ROS under intense cell proliferation (Tsai-Turton and Luderer, 2005). However, glutamate signaling through mGlu receptors remains highly unexplored; studies in the ovary are only descriptive. In the macaque ovary, a strong immunolabeling for mGlu2/3 receptor is present in the oocyte, the theca, and in granulosa cells. No staining was detected in the atretic follicles, corpora albicans, or the stroma (Gill et al., 2008). Likewise, in the rat ovary, the oocyte showed intense staining for mGlu2/3, whereas the corpus luteum was moderately immunoreactive, and the rest of ovarian structures had a very faint signal (Gill and Pulido, 2001). Furthermore, in the rat uterus, anti-mGlu2/3 antibodies show preferential binding to the most superficial layer of the stratified squamous epithelium of the exocervix (Gill and Pulido, 2001). In humans, the presence of estrogens during the first half of the menstrual cycle is associated with predominance in superficial cells in the cervical epithelium. Conversely, with the influence of progesterone after ovulation, this cell type becomes less predominant (Noyes et al., 1975). It is noteworthy mGlu2/3 expression is predominant in proliferating ovarian and uterine structures, which indicates that its production may be cyclically regulated and is also consistent with a differential need for glutamate/glutathione depending on the proliferative status of the tissue. Finally, mGlu4 receptor has also been found in the human cervix and is weakly expressed in the endometrial glands (Chang et al., 2005), whereas the ovary proved to be negative for mGlu4 (Chang et al., 2005).

In summary, most of studies involving mGlu receptors in steroidogenic and reproductive tissues involve only detailed descriptions of their anatomical localization, with little functional data available. The participation of mGlu receptors in events such as steroid hormone production, germinal cell development, and cyclic cell turnover is an exciting field that remains to be fully exploited.

VII. Metabotropic Glutamate Receptors and the Immune Response

Clinical data indicate that elevated plasma concentrations of L-glutamate are associated with immune deficiency, and in vitro assays show that high concentrations of L-glutamate (>100 μM) inhibit mitogen-induced T-cell proliferation (Ferrarese et al., 2001; Lombardi et al., 2001). It is not surprising, then, that immune cells express mGlu receptors; these may well be part, as in the CNS, of an emergency mechanism that is activated once high levels of glutamate are reached.

A. Immune Cell Maturation and Activation

Bone marrow-derived T-cell precursors undergo differentiation in the thymus in a process that involves

regulation of the expression of various membrane proteins. Group I and II mGlu receptors are expressed in whole mouse thymus, isolated thymocytes, and a thymic stromal cell line, as has been demonstrated by both RT-PCR and Western blot analysis (Storto et al., 2000a). Flow cytometry has also shown that mGlu5 receptor expression is induced along thymocyte maturation, whereas mGlu1a had the opposite pattern of expression. The level of mGlu2/3 expression remained unaltered through the different stages of thymocyte maturation (Storto et al., 2000a). A different study showed a positive immunostaining and Western blot of mGlu receptors 2/3, 4, and 5 in rat thymic cells (Rezzani et al., 2003). Moreover, expression of mGlu 2/3, 4, and 5 receptors was abundant in dendritic cells and lymphocytes of the thymic medulla but was weak in lymphocytes of the cortex. After 2 days of treatment with the immunosuppressant agent cyclosporine, a rapid inhibition on the expression of mGlu 2/3, 4, and 5 was induced in the rat thymus, reaching undetectable levels after 3 weeks of treatment (Rezzani et al., 2003).

mGlu receptor expression is not exclusive to young immune cells, in that mature lymphocytes are activated by selective mGlu receptor ligands. When rat peripheral lymphocytes are exposed to the group III mGlu receptor agonist L-AP4, they respond by producing ROS. Moreover, activation of lymphocytes with both an ionotropic receptor agonist [*N*-methyl-D-aspartic acid (NMDA)] and L-AP4 caused a synergistic increase in ROS levels and induced necrotic cell death without activating the proapoptotic caspase-3 pathway that was observed in the presence of NMDA alone (Boldyrev et al., 2004). The majority of circulating lymphocytes express the cell surface protein CD3; treatment with anti-CD3 antibodies can induce apoptosis, a response known as activation-induced cell death. It is noteworthy that L-glutamate has an inhibitory effect on apoptosis induced by anti-CD3 treatment, which is believed to be a consequence of decreased expression of Fas Ligand, an important component of immune cell programmed death mechanisms (Chiocchetti et al., 2006). The mGlu receptor agonists ACPD, quisqualate, DHPG, and (*R*,*S*)-2-chloro-5-hydroxyphenylglycine can mimic this prosurvival effect. The effects of quisqualate were inhibited by the antagonists MPEP, AIDA, and LY367385 (Chiocchetti et al., 2006). These data indicate that both mGlu1 and mGlu5 receptor signaling may be important for the pro-survival effects of glutamate on peripheral lymphocytes.

Whereas resting peripheral T cells are unable to cross the BBB under normal circumstances, access can be gained subsequent to cellular damage in the CNS (such as after an ischemic attack), where they assist neurons against L-glutamate neurotoxicity by inducing changes in microglial phenotype (Pacheco et al., 2007). Differential expression of glutamate receptors upon T-cell activation could be relevant to this process: Pacheco and colleagues (2007) demonstrated that the mGlu5 receptor is present constitutively in human peripheral blood lymphocytes, whereas mGlu1 is expressed only upon activation via the T-cell receptor-CD3 complex (Pacheco et al., 2004). In that specific study, activation of mGlu5 did not trigger the phospholipase C signaling pathway but instead activated adenylate cyclase, thus being presumably mediated by the long splice-variant of mGlu5 termed mGlu5a. On the other hand, only mGlu1 receptor was linked to ERK1/2 activation. Consistent with their differential expression in resting and activated lymphocytes and different signaling pathways, mGlu5 mediates the inhibition of cell proliferation evoked by glutamate, which is reverted by the activation of inducible mGlu1 (Pacheco et al., 2004). In addition, the mGlu5 receptor antagonist MPEP induced a significant increase in IL-6 secretion, whereas the specific mGlu1 receptor antagonist 7-(hydroxyimino)cyclopropa[b]chromen-1α-carboxylate ethyl ester impaired IL-2, IL-6, IL-10, tumor necrosis factor-α, and interferon-γ production (Pacheco et al., 2006). In addition, dendritic cells are able to secrete glutamate when interacting with T cells, a process that seems to be essential for lymphocyte function, because the absence of glutamate led to impaired Th1 (IL-2 and interferon-γ) and proinflammatory (IL-6 and tumor necrosis factor-α) cytokine production. These changes were not associated with a decrease in T-cell proliferation (Pacheco et al., 2006).

B. Autoimmunity

mGlu receptor expression in peripheral tissues seems to be relevant for the development of autoimmune-related disorders. The production of glutamate receptor autoantibodies is involved in the pathophysiology of some paraneoplastic encephalopathies; two patients with Hodgkin's disease represent an interesting case. Although the cancer was in remission, they developed severe cerebellar ataxia and produced mGlu1 autoantibodies that had the ability to block L-glutamate-induced inositol phosphate formation and to induce ataxia when administered intrathecally to mice (Pleasure, 2008). This may be considered circumstantial evidence for the role of mGlu receptors in the initiation or maintenance of central autoimmune disorders; however, it could be relevant in the context of peripheral immune dysregulation. In the case of CNS-specific antigens, these are unlikely to be exposed to the peripheral immune system unless a cerebrovascular infarction or a severe inflammatory condition occurs. mGlu receptors, on the other hand, are widely expressed in the periphery, so one can speculate that they can induce the generation of autoantibodies upon immune dysregulation, resulting in deleterious effects not only in the CNS but also in the periphery.

mGlu4-deficient mice are highly susceptible to experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis (Fallarino et al., 2010). Conversely, increased resistance to EAE is conferred by treatment of

wild-type mice with PHCCC, which can act as an mGlu4-positive allosteric modulator but also as a group I mGlu receptor antagonist (Fallarino et al., 2010). This is in agreement with a previous report showing that long-term L-AP4 treatment increases the recovery rate from EAE in Lewis rats (Besong et al., 2002).

IL-17-producing T helper (Th-17) cells are considered mediators of autoimmunity in multiple sclerosis and EAE, and accumulation of Th-17 cells in the CNS as well as in the periphery is associated with the development of demyelinating plaques (Segal, 2010). Fallarino et al. (2010) show evidence indicating that the absence of mGlu4 in dendritic cells is key to induce a differentiation of T helper cells toward the Th-17 phenotype. They also suggest that activation of mGlu4 receptor (as a result of elevated levels of glutamate produced at the neuroinflammatory status) might exert a protective effect by preventing the unbalance in T helper cells. Such mechanism presents a clear therapeutic potential in this and maybe other demyelinating diseases.

In other autoimmune disorders, such as rheumatoid arthritis, synovial cells (the modified fibroblasts residing between the cartilages of a joint) are surrounded by a fluid rich in inflammation mediators (Flood et al., 2007). Synovial fluid from patients with rheumatoid arthritis and from animal models of arthritis also contain increased levels of L-glutamate, in correlation with elevated concentrations of cytokines (Flood et al., 2007). Only mGlu8, but not mGlu receptors 1 to 7, is expressed in rat cultured synovial fibroblasts. mGlu8 is also present in synovial tissues, with no change in expression in a collagen-induced model of rheumatoid arthritis (Hinoi et al., 2005). In contrast, another study shows that the mGlu4 receptor is expressed in healthy rat patella and menisci and in normal human synovial cells but not in synoviocytes obtained from patients with rheumatoid arthritis or in knee cartilage from patients with osteoarthritis (Flood et al., 2007). However, this potential protective role for the mGlu4 receptor warrants further investigation.

VIII. Metabotropic Glutamate Receptors in Endothelial Function

A. Endothelial Oxidative Damage

Oxidative status of endothelial cells is strongly dependent on glutathione availability (Kevil et al., 2004), and the first and rate-limiting step in glutathione biosynthesis requires L-cysteine, glycine, and L-glutamate, as shown in Fig. 2 (Martin and Teismann, 2009). However, increasing evidence suggests that L-glutamate may not serve only as a metabolic substrate upon oxidative injury: activation of group I mGlu receptors with the agonist DHPG prevents nitric oxide-induced toxicity, DNA degradation, and the progression of membrane phosphatidylserine exposure in endothelial cells from rat brain (Lin and Maiese, 2001), suggesting that glutamatergic signaling trough mGlu1 or mGlu5 receptors may be protective during episodes of acute oxidative damage.

B. Endothelial Cell Barrier

The vascular endothelial cell monolayer regulates transport of macromolecules and fluid between blood and the interstitium, thus acting as a dynamic and semiselective barrier (Wu, 2005). Some organs, such as the CNS structures, the retina, and testes, are further protected from external stimuli by additional selective cellular barriers such as the BBB and blood-retinal barrier (Hosoya and Tachikawa, 2009; Banks, 2010). A variety of stimuli such as physical or inflammatory injury or bioactive compounds can alter the endothelial barrier and increase vessel permeability, thereby compromising organ function (Mehta and Malik, 2006).

L-Glutamate has been shown to have a role in the maintenance of endothelial barrier as inhibition of glutamate transport from the aqueous humor causes an increase in paracellular permeability in the intraocular endothelium/epithelium (Langford et al., 1997). Cell-free supernatants from stimulated polymorphonuclear leukocytes (PMN) significantly altered endothelial permeability, suggesting the presence of soluble PMN-derived mediators. Mass spectrometry analysis of PMN-derived supernatants indicated that glutamate is one of the components regulating human endothelial barrier function (Collard et al., 2002). It is noteworthy that microvascular endothelial cells from human brain (BMVEC) express mGlu receptors 1, 4, and 5 (Collard et al., 2002). Furthermore, group I (DHPG) or III (L-AP4) mGlu receptor agonists significantly increased the paracellular permeability of BMVEC cultured as a monolayer. Similar treatment of human BMVEC with the group I (PHCCC) or III (CPPG) mGlu receptor antagonists attenuated glutamate-mediated increases in paracellular permeability (Collard et al., 2002). In a mouse model of hypoxia, pretreatment with PHCCC or CPPG significantly decreased the permeability of the blood-brain barrier (Collard et al., 2002). Conversely, group I and III mGlu receptor agonists induced an increased permeability both in the hypoxic and normoxic mice (Collard et al., 2002).

Endothelial cells from other sources also express mGlu receptors: microvascular endothelial cells from human skin are positive for mGlu1, mGlu4, and mGlu5 (Collard et al., 2002). mGlu5 immunoreactive material has been found in the lining of blood vessels both in rat and macaque hearts (Gill et al., 1999; Mueller et al., 2003). An mGlu2/3 signal is present in the endothelial lining of the rat respiratory tissues (Gill et al., 2000). Whether these receptors have a role in modulating endothelial oxidative damage, endothelial barrier integrity, or others is yet to be investigated.

IX. Metabotropic Glutamate Receptors in the Muscloskeletal System: a Role in the Maintenance of Bone Mass

Bone cells express glutamate receptors and transporters, and evidence indicates that glutamate might have a role in the maintenance of bone mass under normal or injured conditions (Moriyama and Yamamoto, 2004). Gu and Publicover (2000) reported the expression of mGlu1b receptor in rat femoral osteoblasts, but they were unable to detect mGlu receptor 1a, 2, 3, 4, 5, or 6 by RT-PCR. Blockade of mGlu receptors with (*S*)-α-methyl-4-carboxyphenylglycine resulted in an enlarged glutamate-induced current that resembled the response to NMDA. On the contrary, pretreatment with group I mGlu receptor agonist ACPD reduced the NMDA-induced current.

On the other hand, Hinoi et al. (2001) analyzed all mGlu receptor subtypes in rat cultured calvarial osteoblasts and only mGlu4 and mGlu8 mRNAs were detected independently of the number of days of culture. L-AP4 inhibited the forskolin-induced accumulation of cAMP, an effect that was prevented by the group III mGlu receptor antagonist CPPG.

The mRNA and protein for mGlu6 (but not for mGlu1–5) were identified in rat femoral marrow stromal cells (Foreman et al., 2005). In these cells, which can be classified within the osteoblast lineage, L-glutamate decreases nitric-oxide synthase activity and reduces the levels of intracellular Ca^{2+}. A group III mGlu receptor inhibitor, MAP4, abolished both effects. L-Glutamate-induced membrane hyperpolarization was also sensitive to group III mGlu inhibition (Foreman et al., 2005).

The counterpart to bone formation is mediated by osteoclasts, cells responsible for bone resorption. RT-PCR studies showed the presence of mGlu3, mGlu5, and mGlu8 receptor mRNA in mouse osteoclasts. Immunohistochemistry confirmed the presence of only mGlu8 in mature osteoclasts. (1*S*,3*R*,4*S*)-1-Aminocyclopentane-1,3,4-tricarboxylic acid (a specific agonist of group III mGlu receptors) decreased KCl-evoked secretion of L-glutamate and bone degradation products. This was blocked by the group III receptor antagonist CPPG. Agonists for other mGlu receptors and NMDA receptor did not affect the secretion of glutamate (Morimoto et al., 2006). These authors postulate that upon stimulation, glutamate and bone degradation products are secreted by osteoclasts through transcytosis. Then, the released glutamate exerts an autocrine negative feedback by signaling through mGlu8 receptor, keeping osteoclasts in a suppressed state. In a mutant mouse lacking the vesicular glutamate transporter, such negative feedback would not be effective leading to desuppressive state of osteoclasts, which would cause stimulated bone resorption followed by osteoporosis (Morimoto et al., 2006). These data have relevance to the management and potential drug discovery efforts for pathologic conditions involving increased bone degradation such as osteoporosis.

Other components of the locomotor system also express mGlu receptors: the fibrocartilaginous meniscus and articular cartilage chondrocytes from the adult rat knee express mGlu4 (Flood et al., 2006). Cultured rat costal chondrocytes express the mRNA for mGlu receptors 1, 2, 4, and 8 but not mGlu receptors 3, 5, 6, and 7. In these cells, L-AP4 significantly inhibited the accumulation of cAMP induced by forskolin and parathyroid hormone in a manner sensitive to the group III mGlu receptor antagonist CPPG (Wang et al., 2005).

The role of mGlu receptors in skeletal muscle, unlike enteric muscle, is not yet clear. In muscle cells, the regulation of some ion-transporting systems is critical not only for maintaining the membrane potential but also for water homeostasis. This transport is modulated by inhibitory glutamatergic motoneurons via activation of glutamate receptors located on the sarcolemma. The dipeptide *N*-acetylaspartylglutamate participates in the synaptic transmission as an agonist of ionotropic glutamate receptors and mGlu receptors as well. Nerve transection significantly increases muscle fiber cross-section, but as shown in the rat phrenic muscle, ethylglutamic acid, an mGlu2/3 receptor antagonist had no effect on denervation-induced increase in muscle fiber volume. This indicates that mGlu2/3 may not be involved in the effect of *N*-acetylaspartylglutamate in the volume of skeletal muscle fiber after denervation (Malomuzh et al., 2006).

X. Sensing the World through mGlu Receptors

The role of L-glutamate signaling through mGlu receptors in the context of peripheral sensory cells—i.e., visual, taste, and pain sensory cells—has been previously extensively reviewed (Karim et al., 2001; Neugebauer and Carlton, 2002; Bigiani, 2005; Connaughton, 2005; Shigemura et al., 2009; Yasumatsu et al., 2009). The following sections summarize more recent evidence for a role of mGlu receptors in regulation of sensory organ function and its pharmacological manipulation.

A. Retina

All mGlu receptors, with the exception of mGlu3, have been identified in the retina. Immunohistochemistry and in situ hybridization studies have shown differential expression of mGlu receptors in the different cell layers of retina (Connaughton, 2005). Jensen (2006) used extracellular microelectrodes to study the effects of activation of group II mGlu receptors on the responses of rabbit retinal ganglion cells to a moving light stimulus with responses in these cells shown to be substantially reduced by the group II receptor agonist DCG-IV; this effect was reversed by the group II receptor antagonist ethylglutamic acid. In addition, responses that were direction-selective were prolonged by the use of an acetylcholinesterase inhibitor; this was reversed by DCG-IV,

suggesting that postsynaptic group II mGlu receptors have the potential to influence retinal ganglion cells by inhibiting light-evoked acetylcholine release (Jensen, 2006).

Defects in the gene encoding the mGlu6 receptor can lead to congenital stationary night blindness (CSNB), characterized by myopia and impairment of night vision. It is noteworthy that Zeitz et al. (2007) demonstrated that CSNB-associated mutations in three different domains of mGlu6 receptor interfere with proper protein trafficking to the cell surface (Zeitz et al., 2007). Further to this, Beqollari et al. (2009) investigated how the signaling cascade was affected in a cellular model of CSNB carrying an induced mutation of the mGlu6 receptor (E775K). E775K is incapable of activating G_o and primarily couples to G_i proteins, suggesting that the mGlu6 receptor primarily functions through G_o proteins in retinal ON bipolar cells. An inability of the receptor to activate G_o would result in loss of function and lead to CSNB (Beqollari et al., 2009).

B. Inner Ear

Group I mGlu receptors contribute to the neurotransmission between inner hair cells and afferent neurons in the mammalian cochlea. The group I mGlu receptor agonist DHPG and the ionotropic glutamate receptor agonists α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate and NMDA produced an increase in afferent firing, activation that was reversibly blocked by the group I mGlu receptor antagonist AIDA in a dose-dependent manner (Kleinlogel et al., 1999). Cochlear afferent neurons are sensitive to injury by ischemia and noise exposure; dopamine released from cochlear efferent fibers is believed to exert a protective function by reducing glutamate excitotoxicity on afferent neurons. The group II mGlu receptor agonist APDC dose-dependently increased the release of dopamine in a guinea pig cochlea preparation, whereas group I and III mGlu receptor agonists and antagonists were ineffective. In addition, the $GABA_A$ receptor antagonist bicuculline reversed the effect of APDC, suggesting that the mGlu-mediated increase on dopamine release was due to a disinhibitory mechanism involving local GABAergic fibers (Doleviczényi et al., 2005).

Histochemical studies in human and mouse show that mGlu7 receptor is expressed in hair cells and in spiral ganglion cells of the inner ear (Friedman et al., 2009). Age-related hearing impairment, also known as presbycusis, is the most prevalent sensory impairment in the elderly. Common alleles of GRM7, the gene encoding for mGlu7 receptor, contribute to an individual's risk of developing age-related hearing impairment. Because presbycusis is associated to the loss of sensory cells, polymorphisms in the *mGlu7* gene could possibly be part of a mechanism involving altered susceptibility to glutamate excitotoxicity (Friedman et al., 2009).

C. Pain Sensation

mGlu receptors are involved in various stages of central pain processing (Karim et al., 2001; Varney and Gereau, 2002; Goudet et al., 2009) and interact with TRPV1 receptors, which are involved in the modulation of pain sensation (Hu et al., 2002; Yang and Gereau, 2002). It is noteworthy that mGlu5 receptors expressed on the peripheral terminals of sensory neurons are also involved in nociceptive processes and contribute to the hyperalgesia associated with inflammation (for review, see Neugebauer, 2001). When inflammation was induced in the rat hind paw by administration of Freund's complete adjuvant, hyperalgesia was significantly reduced by intraplantar microinjection, but not by intracerebroventricular or intrathecal microinjection, of the selective mGlu5 receptor antagonist MPEP. Immunohistochemistry confirmed the presence of mGlu5 in peripheral nerve fibers in the naive rat hind paw skin (Walker et al., 2001).

A relevant aspect when dealing with inflammatory pain is treating the inflammation itself. Intraplantar injection of bee venom induces spontaneous nociception, heat, and mechanical hyperalgesia together with an inflammatory response. Local pretreatment with the group I mGlu receptor antagonist AIDA or with the group II and III receptor agonists APCD and L-AP4, respectively, inhibited bee-venom induced spontaneous nociception. Only APCD was able to reduce mechanical hyperalgesia. Group II and III mGlu receptor antagonists proved to reverse the effects induced by the respective agonists, and drug treatments contralateral to the bee venom injection had no effect, suggesting the participation of local mGlu receptors in the induction/reversal of venom-induced spontaneous nociception. It is noteworthy that neither AIDA, APCD, or L-AP4 had an effect on inflammatory swelling (Chen et al., 2010).

Jang et al. (2004) studied the contribution of peripheral mGlu receptors to the development of hyperalgesia in neuropathic pain. They used a rat model involving spinal nerve lesion (SNL) preceded by dorsal rhizotomy, which is performed to avoid the potential central effects induced by SNL. By analyzing the paw withdrawal threshold to increasing mechanical stimuli they show that blockade of peripheral group I mGlu receptors with the antagonist D,L-2-amino-3-phosphonopropionic acid prevents the induction of neuropathic pain behavior. When D,L-2-amino-3-phosphonopropionic acid is administered locally to the hind paw before SNL, it delays the onset of SNL-induced mechanical hyperalgesia. Conversely, intraplantar injection of the group II mGlu receptor agonist APDC also delays the onset of mechanical hyperalgesia, suggesting that activation of peripheral group II mGlu receptors prevents the induction of neuropathic pain behavior (Jang et al., 2004).

XI. Metabotropic Glutamate Receptors Commanding Cell Proliferation, Differentiation, and Transformation

A. Embryonic Development

The involvement of glutamate in early CNS development has been extensively investigated. Double-knockout mice lacking the glutamate transporters GLAST and GLT1 show multiple brain defects and perinatal mortality (Matsugami et al., 2006). In addition, mGlu3 receptor is known to induce differentiation of neural stem cells (Ciceroni et al., 2010). Less is known about glutamatergic signaling in immature peripheral tissues; activation of mGlu receptors has been addressed using mainly in vitro or ex vivo models of embryonic development.

Embryonic stem (ES) cells can be grown in vitro as undifferentiated, self-renewing cells in the presence of leukemia inhibitory factor, a member of the interleukin-6 family of cytokines (Cappuccio et al., 2005; Spinsanti et al., 2006). Cultured mouse ES cells maintained under this pluripotent condition express mGlu5 receptors, whereas the mRNA of all other mGlu receptor subtypes was not detectable by RT-PCR. The presence of mGlu5 was confirmed by Western blot and flow cytometry (Cappuccio et al., 2005). An increase on intracellular Ca^{2+} was produced when ES cells were treated with quisqualate in the absence of extracellular L-glutamate, which indicates that mGlu5 was activated by endogenous glutamate. Blockade of mGlu5 receptors with MPEP or antisense-induced knockdown of mGlu5 decreased the expression of transcription factors such as c-Myc, important to maintain ES cell-undifferentiated state. Likewise, exposure of ES cells to MPEP reduced alkaline phosphatase activity, another marker of undifferentiated state in ES cells (Cappuccio et al., 2005). Endogenous activation of mGlu5 sustains the increase in c-Myc induced by leukemia inhibitory factor in embryonic stem cells by inhibiting glycogen synthase kinase-3b and phosphatidylinositol 3-kinase. These two effects result from the activation of protein kinase C (Spinsanti et al., 2006).

On the other hand, when cultured ES cells form aggregates or embryoid bodies (EBs), which differentiate in a way that resembles embryogenesis, they start losing mGlu5 and begin to express mGlu4 receptor. This switch could be critical for further development, because L-AP4 induces an increase on mRNA for brachyury and H19 (a mesoderm-specific transcription factor and a noncoding RNA produced by endoderm, respectively), and a decrease on the expression of the mRNA for fibroblast-growth factor-5, a more primordial ectoderm marker. The mGlu4 receptor antagonist (*R*,*S*)-α-methylserine-*O*-phosphate prevented these effects. In addition, when EBs were incubated in a medium that induces an enrichment of the culture on neural precursors, L-AP4 increased the expression of the neural markers nestin and Dlx-2, showing that mGlu4 affects cell differentiation in a context-dependent manner (Cappuccio et al., 2006).

During embryonic development of the spine and long bones, mesenchymal stem cells differentiate by forming a cartilaginous structure that then undergoes ossification to finally induce bone formation (Karsenty, 2003). L-Glutamate seems to be a key component of chondrocyte developmental maturation, because ex vivo data indicate that L-glutamate suppresses chondral mineralization and induces apoptosis in cultured embryonic mouse metatarsals (Wang et al., 2006). In the same model, mineralization is also greatly inhibited by the group III mGlu receptor agonist L-AP4. This effect was independent of apoptosis and also sensitive to CPPG. The metatarsal total length remained unchanged. Similar to L-AP4, a group II mGlu receptor agonist (DCG-IV) was more effective in inhibiting the mineralization than a group I mGlu receptor agonist (DHPG), whereas none of the iGlu receptor agonists (α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate, kainate, or NMDA) drastically affected the mineralization (Wang et al., 2005).

B. Tumoral Growth

Plasma levels of L-glutamate are generally elevated in patients with carcinoma (Dröge et al., 1987) and seem to correlate with an impairment in immune function. Furthermore, tumor cells show decreased motility and invasiveness upon administration of ionotropic glutamate receptor antagonists (Rzeski et al., 2001). mGlu receptors are also expressed in several cell lines derived from human tumors, including neuroblastoma, rhabdomyosarcoma/medulloblastoma, thyroid carcinoma, lung carcinoma, astrocytoma, multiple myeloma, glioma, colon adenocarcinoma, T-cell leukemia, and breast carcinoma (for review, see Stepulak et al., 2009). Massive mGlu receptor expression is sometimes seen in transformed tissues; this is the case for mGlu3 mRNA, which is increased by 5-fold in aldosterone-producing adenomas compared with normal human adrenal glands (Ye et al., 2007). The implications of such overexpression remain unknown; however, increasing data suggest that mGlu receptors could be novel targets for the treatment of especially aggressive or chemotherapy-resistant tumors (see Tables 3, 4, and 5 for a summary).

A good amount of evidence implicates mGlu1 in the pathogenesis of melanoma. Human melanoma biopsies and cell lines, but not healthy nevi or melanocytes, express mGlu1 (Pollock et al., 2003). In the TG-3 mouse, a transgenic mouse model that is predisposed to develop multiple melanomas, expression of mGlu1 in skin tumors is sufficient for melanoma development in vivo (Pollock et al., 2003). Cell lines derived from TG-3 mouse melanoma lesions express the two members of group I, mGlu receptors 1 and 5. To explore the contribution of mGlu5 to melanoma development, mGlu5-null mice were crossed with TG-3 mice. The resulting offspring still developed tumors with onset, progression, and me-

TABLE 3

Site of action for pharmacological agents acting on group I mGlu receptors in the periphery

This table does not include studies performed in sensory cells.

Agent	Site of Action	Effect	References
Agonists			
ACPD[a]	Adrenal glands	Increased secretion of catecholamines	González et al., 1998
	Testis	Increased inositol phospholipid hydrolysis	Storto et al., 2001
	Enteric neurons	Activation of depolarizing response	
	Peripheral T cells	Increased survival to apoptosis induced by anti-CD3	Ren et al., 2000
	Hepatocytes	Increased inositol monophosphate formation and hypoxia-induced cell damage	Chiocchetti et al., 2006
	Osteoblasts	Reduced NMDA-induced current	Storto et al., 2000b; Sureda et al., 1997 Gu and Publicover, 2000
CHPG[b]	Peripheral T cells	Increased survival to apoptosis induced by anti-CD3	Chiocchetti et al., 2006; Pacheco et al., 2004
	Enteric neurons	Activation of depolarizing response	Liu and Kirchgessner, 2000
	Enteric glia	Increased expression of c-Fos and activation of ERK1/2	Nasser et al., 2007
DHPG	Chromaffin cells	Increased secretion of catecholamines	Arce et al., 2004
	Pancreatic β cells	Increased release of insulin at low glucose concentrations	Brice et al., 2002
	Duodenum	Increased intracellular pH	Akiba et al., 2009
	Enteric neurons	mGlu5 internalization; activation of depolarization; suppression of slow EPSPs	Liu and Kirchgessner, 2000; Ren et al., 1999; 2000
	Peripheral T cells	Increased survival to apoptosis induced by anti-CD3	Chiocchetti et al., 2006; Pacheco et al., 2004
	Endothelial cells	Increased paracellular permeability; prevents oxidative damage	Collard et al., 2002; Lin and Maiese, 2001
	Cartilage	Decreased mineralization	Wang et al., 2005
	Melanocytes	Impaired viability in confluent cultures; proliferation in subconfluent cultures	Frati et al., 2000
	Tumor cells	Increased cell migration, invasion, and adhesion	Park et al., 2007
Quisqualate	Hepatocytes	Increased inositol monophosphate formation and hypoxia-induced cell damage	Storto et al., 2000b; Sureda et al., 1997
	Heart	Increased PLC activity	Iglesias et al., 2007
	Melanocytes	Impaired viability in confluent cultures; proliferation in subconfluent cultures	Frati et al., 2000
	Stem cells	Increased intracellular Ca^{2+} levels	Cappuccio et al., 2005
	Peripheral T cells	Increased survival to apoptosis induced by anti-CD3	Chiocchetti et al., 2006
Antagonists			
AIDA	Duodenum	Decreases glutamate-induced alkalinization	Akiba et al., 2009
	Ileum	Suppression of DHPG-induced depolarizing response	Liu and Kirchgessner, 2000
	Peripheral T cells	Inhibition of prosurvival effect induced by quisqualate	Chiocchetti et al., 2006
BAY36-7620[c]	Tumor cells	Reduction in cell proliferation	Namkoong et al., 2007
CPCCOEt[c]	Peripheral T cells	Impaired cytokine production	Pacheco et al., 2006
	Testis	Inhibition of the ACPD-induced inositol phospholipid hydrolysis	Storto et al., 2001
LY367385[c]	Tumor cells	Reduction in cell proliferation	Namkoong et al., 2007
MPEP[b]	Peripheral T cells	Increased IL-6 production and inhibition of Glu-induced survival to apoptosis	Chiocchetti et al., 2006; Pacheco et al., 2006
	Testis	Inhibition of the ACPD-induced inositol phospholipid hydrolysis	Storto et al., 2001
	Pancreas	Inhibition of Glu-induced insulin secretion and of glucagon response to insulin	Storto et al., 2006
	Hepatocytes/ liver	Protection against hypoxia-, acetaminophen-, and LPS-induced damage	Jesse et al., 2009; Storto et al., 2000b, 2003
	Enteric neurons	Reduction of glutamate-induced depolarizing response	Liu and Kirchgessner, 2000
	Melanocytes	Prevention of quisqualate-induced effects	Frati et al., 2000
	Stem cells	Reduction in markers of undifferentiated state	Cappuccio et al., 2005; Spinsanti et al., 2006
	Tumor cells	Inhibition of DHPG-induced cell migration, invasion, and adhesion	Park et al., 2007
PHCCC[d]	Endothelial cells	Attenuated glutamate-mediated increases in paracellular permeability	Collard et al., 2002
	Tumor cells	Reduction of proliferation in vitro and tumor mass growth in vivo	Namkoong et al., 2007
S-4-CPG	Enteric neurons	Reduction of DHPG-induced depolarization; suppression of Glu effect on EPSPs	Hu et al., 1999; Liu and Kirchgessner, 2000; Ren et al., 1999; 2000
	Enteric glia	Abolished intracellular signaling induced by CHPG	Nasser et al., 2007

CHPG, (*R*,*S*)-2-chloro-5-hydroxyphenylglycine; CPCCOEt, 7-(hydroxyimino)cyclopropa[b]chromen-1α-carboxylate ethyl ester; Quisqualate, (L)-(+)-α-amino-3,5-dioxo-1,2,4-oxadiazolidine-2-propanoic acid; S-4-CPG, (*S*)-4-carboxyphenylglycine.

[a] Also activates mGlu receptors from group II.

[b] Specific for mGlu5 receptor.

[c] Specific for mGlu1 receptor.

[d] Also activates mGlu4 receptor.

tastasis very close to that described for TG-3, indicating that mGlu1 can promote melanocyte transformation independently of mGlu5 expression (Marín et al., 2005).

Shin et al. (2008) also showed that immortalized melanocytes express mGlu1 receptor whereas nontransformed melanocytes do not; in addition, the presence of mGlu1 was associated with phorbol ester and anchorage independence, indicating that mGlu1 expression was linked to a higher tumorigenic capacity. mGlu1-expressing immortalized melanocytes are tumorigenic in mice,

TABLE 4

Site of action for pharmacological agents acting on group II and group III mGlu receptors in the periphery

This table does not include studies performed in sensory cells.

Agent	Site of Action	Effect	References
Group II Agonists			
4C3HPG	Thymic cell line	Reduction of forskolin-induced increase of cAMP	Storto et al., 2000a
APCD	Thymic cell line	Reduction of forskolin-induced increase of cAMP	Storto et al., 2000a
DCG-IV	Cartilage	Inhibited mineralization	Wang et al., 2005
L-CCG-1	Pancreatic β cells	Increased release of insulin	Brice et al., 2002
Group II antagonists			
LY341495	Tumor cells	Inhibition of cell proliferation and number of tumor aggregates	Arcella et al., 2005; D'Onofrio et al., 2003; Iacovelli et al., 2006
PCCG-4	Thymic cell line	Inhibition of 4C3HPG-induced effect	Storto et al., 2000a
Group III Agonists			
ACPT-I	Osteoclasts	Reduction in KCl-evoked secretion of glutamate and bone degradation products	Morimoto et al., 2006
	Pancreatic cells	Inhibition of glucagon secretion at low concentrations of glucose	Uehara et al., 2004
AMN082[a]	Colon	Increased fecal water content in vivo and electrolyte secretion in vitro	Julio-Pieper et al., 2010
DCPG	Colon	Increased motility	Tong and Kirchgessner, 2003
	Pancreatic cells	Inhibition of glucagon secretion at low concentrations of glucose	Uehara et al., 2004
L-AP4	Duodenum	Increased intracellular pH and mucus gel thickness	Akiba et al., 2009
	Tumor cells	Increased cell invasiveness	Chang et al., 2005
	Pancreatic β cells	Increased release of insulin at low concentrations of glucose	Brice et al., 2002
	Lymphocytes	Production of ROS	Boldyrev et al., 2004
	Endothelial cells	Increased paracellular permeability	Collard et al., 2002
	Osteoblasts	Inhibited accumulation of cAMP induced by forskolin and parathyroid hormone	Hinoi et al., 2001
	Cartilage	Inhibited mineralization	Wang et al., 2005
	Embryonic bodies	Increased expression of markers for cell differentiation	Cappuccio et al., 2006
PPG	Colon	Increased motility	Tong and Kirchgessner, 2003
	Pancreatic cells	Inhibition of glucagon secretion at low concentrations of glucose	Uehara et al., 2004
Group III Antagonists			
CPPG	Colon	Inhibits motility and PPG-induced increase in motility	Tong and Kirchgessner, 2003
	Endothelial cells	Attenuated glutamate-mediated increases in paracellular permeability	Collard et al., 2002
	Osteoblasts	Prevention of L-AP4 effect	Hinoi et al., 2001
	Osteoclasts	Blockade of effects induced by ACPT-I	Morimoto et al., 2006
	Pancreatic cells	Prevention of Glu-induced inhibition of glucagon secretion	Uehara et al., 2004
MAP4	Duodenum	Prevention of glutamate-induced alkalinization and mucus secretion	Akiba et al., 2009
	Bone stromal cells	Inhibition of Glu-induced reduction of NOS activity and intracellular Ca^{2+} levels	Foreman et al., 2005
MSOP	Embryonic bodies	Inhibition of L-AP4-induced expression of markers for cell differentiation	Cappuccio et al., 2006

ACPT-I, (1*S*,3*R*,4*S*)-1-aminocyclopentane-1,3,4-tricarboxylic acid; L-CCG-1, (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)glycine; MSOP, (*R*,*S*)-α-methylserine-*O*-phosphate; NOS, nitric-oxide synthase; PCCG-4, (2*S*,1′*S*,2′*S*,2′*R*)-2-(2′-carboxy-3′phenylcyclopropyl)glycine; PPG, (*R*,*S*)-phosphonophenylglycine.

[a] Specific for mGlu7 receptor.

and continuous expression of the receptor is required for maintenance of tumorigenicity as demonstrated by small interfering RNA studies (Shin et al., 2008). More recently, human melanoma cells were shown not only to express mGlu1 but also to release high levels of L-glutamate (Namkoong et al., 2007). Treatment of mGlu1-expressing human melanoma cells with the mGlu1 receptor antagonists LY367385 or (3a*S*,6a*S*)-hexahydro-5-methylene-6a-(2-naphthalenylmethyl)-1*H*-cyclopenta[c]furan-1-one (BAY36-7620) induced a reduction in cell proliferation. The same result was obtained with the glutamate release inhibitor and glutamate transporter activator riluzole (Namkoong et al., 2007). It is noteworthy that riluzole also decreased tumor growth by 50% in mice receiving a human melanoma cell graft (Namkoong et al., 2007).

Pharmacological blockade of mGlu3 receptors reduces cell proliferation and mitogen –activated protein kinase activation in cultured human glioma explants or glioma cell lines that express mGlu3 (D'Onofrio et al., 2003). Furthermore, systemic administration of the mGlu2/3 receptor antagonist, (2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid (LY341495), inhibits the growth of glioma cells implanted either under the skin or inside the brain parenchima of nude mice. Treatment with LY341495 also significantly reduced the number of Ki-67-positive tumor cells (Arcella et al., 2005) and tumor cell aggregates (Ciceroni et al., 2008) in the brains of these mice.

Chang et al. (2005) studied the expression of mGlu4 receptor in several healthy and transformed human tissues. mGlu4 receptor expression was identified in 68% of colorectal carcinomas, 50% of laryngeal carcinomas, and 46% of breast carcinomas. In the case of colorectal carcinoma, overexpression of mGlu4 was associated with poor prognosis (Chang et al., 2005), and cell lines derived from human colorectal carcinomas showed increased cell invasiveness when treated with L-AP4 (Chang et al., 2005). In another study, comparative proteomics were used to characterize a human colon cancer

TABLE 5
Disorders in which targeting mGlu receptors may be beneficial

Disorder/pathological condition	Desired Effect	Potential Targets	References
Constipation	Increase in fecal water content	mGlu7 activation	Julio-Pieper et al., 2010
	Increase in colonic motility	mGlu8 activation	Tong and Kirchgessner, 2003
Diabetes	Reduction of glucagon production	mGlu4 activation	Uehara et al., 2004
Duodenal ulcer	Increase in pH, mucus gel thickness	mGlu1, mGlu5 and mGlu4 activation	Akiba et al., 2009
Medulloblastoma	Reduction in cell proliferation	mGlu4 activation	Iacovelli et al., 2006
Osteoporosis	Decrease in bone resorption	mGlu8 activation	Morimoto et al., 2006
Brain glioma	Reduction in cell proliferation	mGlu2/3 inhibition	D'Onofrio et al., 2003
Colorectal carcinoma	Decrease in cell survival and invasiveness	mGlu4 inhibition	Chang et al., 2005
	Improved response to other chemotherapies	mGlu4 inhibition	Yoo et al., 2004
Diabetes (type II)	Reduction of insulinemia	mGlu5 inhibition	Storto et al., 2006
Drug induced liver damage	Decrease in necrosis and production of ROS	mGlu5 inhibition	Storto et al., 2003
Gastroesophageal reflux[a]	Decrease in frequency and intensity of reflux episodes	mGlu5 inhibition	Frisby et al., 2005; Keywood et al., 2009
Hypoxia induced liver damage	Decrease in cell damage and death	mGlu5 inhibition	Storto et al., 2004
Melanoma	Reduction in cell proliferation	mGlu1 inhibition	Shin et al., 2008
Oral squamous cell carcinoma	Decrease in cell migration and invasiveness	mGlu5 inhibition	Park et al., 2007
Pruritus	Prevention or treatment of itching	mGlu1 inhibition	Gasparini and Spooren, 2007
Sepsis	Decrease in proinflammatory cytokines	mGlu1 inhibition	Pacheco et al., 2006

[a] In phase I clinical trial.

cell line that was resistant to 5-fluorouracil (5-FU; a common chemotherapy agent). 5-FU-resistant cells were found to overexpress mGlu4 in comparison with the parental cancer cells (Yoo et al., 2004). In the presence of 5-FU, cell survival was increased by the group III mGlu receptor agonist L-AP4 in the nonresistant parent cancer cells; conversely, survival was synergically decreased by 5-FU and the group III receptor antagonist MAP4 in 5-FU-resistant cells (Yoo et al., 2004). It is noteworthy that 5-FU down-regulated mGlu4 expression, and MAP4 had a dose-dependent cytotoxic effect in both cell lines (Yoo et al., 2004).

In contrast to the above evidence, the expression of mGlu4 receptors seems to be inversely related to the severity of human medulloblastoma. After scoring the extent of immunoreactivity for mGlu4 in human biopsies of medulloblastoma, the absence of spinal metastases, cerebrospinal fluid spread, and tumor recurrence as well as the survival of patients were all shown to be associated with high levels of mGlu4 immunoreactivity. Treatment with PHCCC (which is considered a group I mGlu receptor antagonist but can also act as a positive allosteric modulator of mGlu4 receptor) reduced the proliferation of cultured medulloblastoma cells and inhibited the growth of medulloblastoma implants in mice. In addition, subcutaneous or intracranial injections of PHCCC during the first week of life reduced the incidence of medulloblastoma from 85 to 28% in a mutant mouse model known to develop the disease upon X-ray irradiation. This indicates that activation of mGlu4 receptors also affects early events in tumorigenesis (Iacovelli et al., 2006).

In the case of human oral squamous cell carcinoma, strong immunoreactivity for mGlu5 was also associated with a decreased survival rate (Park et al., 2007). The mGlu5 receptor agonist DHPG increased tumor cell migration, invasion, and adhesion in human tongue cancer cells, an effect that was reversed by the mGlu5 receptor antagonist MPEP (Park et al., 2007).

C. Other Disorders Involving Pathological Cell Proliferation/Differentiation

In the retinal epithelium, the pathological transition of epithelial cells to a mesenchymal phenotype, a condition known as proliferative vitreoretinopathy, can lead to retinal detachment and the loss of vision. Abnormal proliferation and migration of retinal pigment epithelium cells play a key role in the development of this retinopathy. It has been shown that glutamate stimulates human retinal pigment epithelium cell proliferation in vitro as well as ERK and cAMP response element-binding protein phosphorylation. These effects can be mimicked by the group I mGlu receptor agonist ACPD and prevented by the receptor inhibitor (*S*)-α-methyl-4-carboxyphenylglycine. In addition, inhibition of the MEK/ERK pathway prevents ACPD-induced cell proliferation (García et al., 2008). These authors suggest that retinal detachment or an injury to the blood-retinal-barrier may allow the passage of high levels of glutamate to the retina epithelium, thus contributing to the development of proliferative vitreoretinopathy.

XII. Other Tissues Expressing Metabotropic Glutamate Receptors

In the adult rat skin, mGlu receptor expression would be limited to particular subpopulations of basal keratinocytes, according to Genever et al. (1999). Abundant mGlu1a immunoreactivity was described in general for keratinocytes, whereas mGlu2/3 expression was less widespread and restricted to a small subpopulation of basal keratinocytes (Genever et al., 1999). Cultured human melanocytes obtained from neonatal foreskin express mGlu5, as shown by RT-PCR, immunocytochem-

istry, and Western blot analysis. The group I mGlu receptor agonists DHPG and quisqualate increased [^{3}H]thymidine incorporation and melanocyte proliferation in subconfluent cultures but impaired cell viability in confluent cultures. The mGlu5 receptor antagonist MPEP prevented both effects. Agonists of other mGlu receptor subtypes or selective agonists of ionotropic glutamate receptors did not affect melanocyte proliferation or viability (Frati et al., 2000).

In the heart, most of studies involving mGlu receptors are detailed descriptions of their anatomical localization. In the rat heart mGlu1a, mGlu2/3, and mGlu5 are localized preferentially in the atrial nerve terminals, ganglion cells, and elements of the conducting system. mGlu5 was the only mGlu receptor located in the intercalated disks of the myocardium (Gill et al., 1999). In the macaque heart, mGlu2/3 and -5 were found in the myocardial nerve fibers, atrial intramural ganglia and myocytes, ventricular and submyocardial myocytes, Purkinje fibers, and bundle of Hiss (Mueller et al., 2003). In the human heart, mGlu receptors 1 and 5 but not mGlu2/3 were found in atrial intramural ganglia, atrial and ventricular cardiocytes, and bundle of Hiss (Gill et al., 2007). In addition, the components of mGlu/PLC pathway have been studied in the pregnant rat heart (Iglesias et al., 2006). It is noteworthy that maternal intake of caffeine during rat gestation causes downregulation of mGlu1 protein level in the fetus heart and a loss of responsivity to several mGlu receptor agonists in membrane preparations isolated from the heart of the fetus (Iglesias et al., 2006). Pregnancy itself seems not to affect mGlu-associated PLC pathway in that L-glutamate- or quisqualate-mediated PLC activity was not modified by the pregnant or nonpregnant state (Iglesias et al., 2007).

Normal human gall bladder, breast tissue, and laryngeal epithelium have positive staining for mGlu4 (Chang et al., 2005). A focal expression can also be detected in the bronchus but not in the alveoli (Chang et al., 2005). In the rat, anti-mGlu2/3 signal was distributed in the endothelial lining and more intensely in the bronchiolar and alveolar epithelial lining (Gill et al., 2000). Unfortunately, none of these receptor localizations have been further explored. Likewise, human odontoblasts, the cells secreting dentin in the tooth, are immunoreactive for mGlu5 (Kim et al., 2009). Whether the receptor function is related to dentin production or to the transduction of nociceptive signals from neurons in the pulp remains unknown.

XIII. Intracellular Signaling Driven by Metabotropic Glutamate Receptors in Non-Neural Tissues

In the CNS, activation of mGlu receptors from group I, II, or III is associated with well described intracellular signaling pathways, as shown in Fig. 1 (Conn, 2003; Niswender and Conn, 2010). With some exceptions, the same pathways seem to be true for mGlu receptors localized in non-neural tissues.

Centrally, group I mGlu receptors stimulate the formation of inositol 1,4,5-triphosphate and diacylglycerol (Conn, 2003; Niswender and Conn, 2010). In rat hepatocytes and human melanocytes, the agonist quisqualate activates phosphoinositide hydrolysis (Sureda et al., 1997; Frati et al., 2000). Likewise, in mouse embryonic stem cells, the addition of quisqualate induces an increase in intracellular Ca^{2+} levels (Cappuccio et al., 2005). On the other hand, in human peripheral blood lymphocytes, activation of mGlu5 receptor triggered adenylate cyclase but not phospholipase C signaling pathway, and only the mGlu1 receptor was linked to ERK1/2 activation, showing differential signaling driven by the two members of group I (Pacheco et al., 2004). In addition, stimulation of group I mGlu receptors increases the expression of c-jun and c-fos in Jurkat cells, an immortalized T-cell line (Miglio et al., 2005).

In bovine chromaffin cells, adrenaline secretion elicited by group I mGlu receptor activation seems to be coupled to calcium mobilization from intracellular stores via inositol triphosphate synthesis. On the other hand, noradrenaline secretion seems to be mediated by both calcium mobilization from intracellular stores and from calcium entry from the extracellular space (Arce et al., 2004). As for lymphocytes, differential signaling seems to be the case for the two subtypes of receptor because mGlu5 receptor would be responsible for extracellular Ca^{2+}-independent catecholamine release, whereas Ca^{2+}-dependent noradrenaline release seems to be mediated by mGlu1a (Arce et al., 2004).

Metabotropic glutamate receptors from groups II and III induce a decrease on the intracellular levels of cAMP in the CNS (Conn, 2003; Niswender and Conn, 2010). In agreement with that, rat osteoblasts treated with the group III receptor agonist L-AP4 show an inhibition in the forskolin-induced accumulation of cAMP, an effect that was prevented by the antagonist CPPG (Hinoi et al., 2001). In a mouse thymic stromal cell line, but not in isolated thymocytes, the mGlu2/3 receptor agonists APDC and 4C3HPG reduced the stimulation of cAMP by forskolin. The effect of 4C3HPG was prevented by the receptor antagonist (2*S*,1′*S*,2′*S*,2′*R*)-2-(2′-carboxy-3′phenylcyclopropyl)glycine (Storto et al., 2000a). Pretreatment of marrow stromal cells with the group III mGlu receptor antagonist MAP4 prevented L-glutamate-dependent increase on nitric-oxide synthase activity and intracellular Ca^{2+} levels but did not significantly alter the rate of cAMP production (Foreman et al., 2005).

XIV. Concluding Remarks

L-Glutamate is produced by a great variety of the peripheral tissues in both health and disease. Like other components of the glutamatergic system, mGlu recep-

tors also have a widespread distribution outside the CNS, including cells that do not have a neuronal phenotype (see Fig. 3). Analysis of the recent literature reveals an extraordinary potential, particularly for group I and III mGlu receptors in the treatment of peripheral disorders of the most diverse nature, such as endocrine dysregulation, aberrant cell proliferation, and gastrointestinal disorders (see Table 5). The significance of these findings is that pharmacological tools originally designed for mGlu receptors in the CNS may also be directed toward new disease targets in the periphery. In particular, mGlu4 receptor seems to be the most widely expressed (or at least the most extensively investigated; see Table 2) in the periphery. At the same time, promising data indicate that targeting mGlu4 receptor could be beneficial in conditions such as type II diabetes as well as different types of cancer and autoimmune disorders. However, the ubiquity of this and other mGlu receptors is without doubt a disadvantage in the development of innovative therapies, and multiple side effects can be anticipated. As an example, preclinical studies show that activation of mGlu4 could be helpful in the treatment of medulloblastoma, whereas in other malignancies, such as brain glioma or colorectal carcinoma, the opposite approach (i.e., mGlu4 inhibition) seems to have a beneficial effect (see Tables 4 and 5). When translated into the clinic, such pharmacological approaches could enhance the invasiveness or growth of other types of tumor present in the same patient or even increase the risk of malignant transformation of healthy tissues. Likewise, Akiba et al. (2009) have shown that activation of group I mGlu receptors may be helpful in the treatment of duodenal ulcers by preventing cellular injury induced by acid damage. But from the in vitro data reported by Pacheco et al. (2006), it can be extrapolated that mGlu1 activation could enhance the production of proinflammatory cytokines, a nondesired effect in the treatment of erosive GI conditions. Advances in the development of positive and negative allosteric modulators have been a major breakthrough in the therapeutic po-

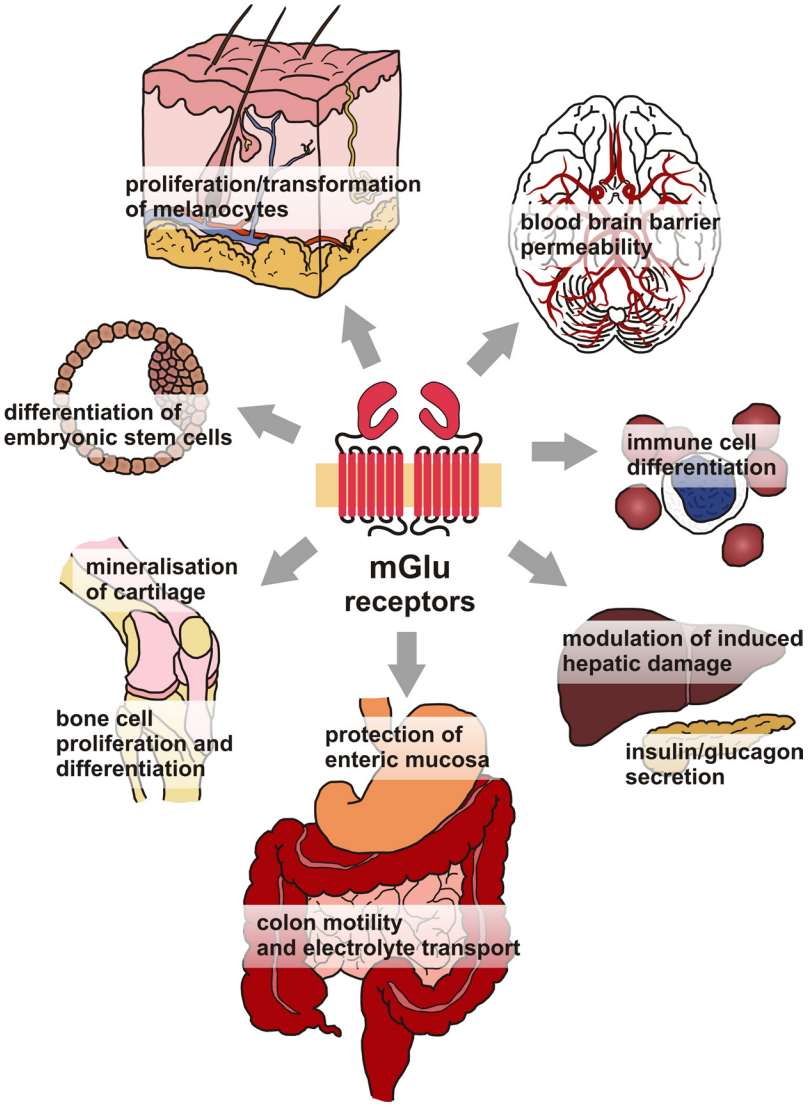

FIG. 3. Summary of roles of mGlu receptors in peripheral tissues.

tential of glutamate ligands. One of the advantages of this approach is that receptor activation only occurs at sites where the endogenous ligand is present, thus minimizing the risk of side effects (Gasparini and Spooren, 2007; Conn et al., 2009; Gregory et al., 2011).

The existence of biomarkers for CNS-targeted compounds (i.e., noticeable changes in the periphery that provide evidence as to whether a particular drug is having an effect) is a valuable tool in drug development (Gomez-Mancilla et al., 2005; Wagner, 2008). The fact that peripheral tissues also respond to mGlu receptor activation has a clear application in this area. Rapid changes in plasma insulin levels or in colonic motility could represent readily measurable parameters when evaluating group I or III mGlu receptor modulators for CNS applications, respectively.

Increasing efforts are being undertaken with the aim of producing mGlu receptor-based pharmacological treatments for neurologic or psychiatric disorders. Storto et al. (2006) conclude in their work that, because mGlu5 receptor activation regulates the secretion of pancreatic hormones, drugs that are currently under development for CNS disorders may affect glucose homeostasis. In the same line of thought, liver damage, constipation, and osteoporosis could represent other side effects for this type of compound. Conversely, it is valid to speculate that long-term mGlu-based treatments for peripheral disorders, such as diabetes, might have side effects affecting the CNS. Nevertheless, it is also possible that targeting mGlu receptors could be useful in the treatment of disorders involving central components together with dysfunction in peripheral organs, such as irritable bowel syndrome or peptic/duodenal ulcer, which can be comorbid with mood disorders, or neoplastic processes, which can be accompanied by a great deal of stress and anxiety. Finally, the elucidation of tissue-specific elements regulating mGlu receptor signaling might aid in the development of more specific therapeutic approaches and guide our understanding of side effects generated from centrally acting mGlu ligands.

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Authorship Contributions

Wrote or contributed to the writing of the manuscript: Julio-Pieper, Flor, Dinan, and Cryan.

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