MINI REVIEW

From biological gastroenterology to fundamental neurosciences: How studies in gastric emptying have led to the discovery of a new mechanism of neuronal functioning

De la gastroentérologie biologique aux neurosciences fondamentales: comment des études sur la vidange gastrique ont conduit à la découverte d’un nouveau mécanisme de fonctionnement neuronal

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Summary Gastric emptying undergoes complex regulation by the nervous system, which organizes in particular the inhibition of duodenum motility after a rise in intra-gastric pressure: the gastro-duodenal inhibitory reflex. It was first shown in mammals that this reflex could be organized by a sympathetic ganglion, the coeliac plexus. The excitation of gastric mechanosensitive fibres leads in this ganglion to the release of a neurotransmitter, which in turn activates ganglionic neurones leading to inhibition of the duodenum contractions. It rapidly became apparent that this reflex presented striking properties since it was organized in the absence of action potentials along the nerve fibres. Then it was shown that the neurotransmitter released in the coeliac plexus was gaseous: nitric oxide (NO). The nature of the mechanism conducting, without action potentials, the excitation along the nerve fibres was recently determined. This mechanism necessitates the integrity of particular areas of the neuronal membrane (the lipid rafts) and the activation in cascade of the following second messenger sequence: ceramide, calcium, NO and guanosine cyclic monophosphate (c-GMP). These results show how studies in biological gastroenterology have led to the rethinking of one of the central dogmas in neuroscience according to which excitation is only conducted along the nerves by the action potential. © 2010 Elsevier Masson SAS. All rights reserved.

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Résumé La vidange gastrique est régulée de manière complexe par le système nerveux. Celui-ci organise en particulier l’inhibition de la motricité duodénale après une augmentation de pression intragastrique : le réflexe gastro-duodénal inhibiteur. Il a initialement été montré chez le mammifère que ce réflexe peut être organisé par un ganglion sympathique, le péristomé. L’excitation des fibres gastriques mécanosensibles provoque la libération dans ce ganglion d’un neuromédiateur qui va activer des neurones ganglionnaires provoquant ainsi une inhibition du duodénum. Il est rapidement apparu que ce réflexe possède des propriétés atypiques puisqu’il est organisé en l’absence de potentiel d’action le long des fibres nerveuses. Puis il a été démontré que le neuromédiateur libéré dans le péristomé coeliaque est gaseux : le monoxyde d’azote. La nature du mécanisme conduisant, sans potentiel d’action, l’excitation le long des fibres nerveuses a été déterminée récemment. Ce mécanisme nécessite l’intégrité de zones particulières de la membrane (les rafts lipidiques) et l’activation en cascade de la séquence de seconds messagers suivants : céramide, calcium, monoxyde d’azote et guanosine monophosphate cyclique. Ces résultats montrent comment des études en gastroentérologie biologique ont débouché en cause d’un des dogmes majeurs des neurosciences qui stipule que l’excitation est uniquement transmise le long des nerfs par le potentiel d’action.

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Introduction

The involvement of peripheral nervous centres in the regulation of gastric emptying has now been studied for 30 years and has brought very original results in the field of biological gastroenterology. Some of these studies have been performed using in vitro preparations in the mammal consisting of the stomach and duodenum connected to a sympathetic ganglion, the coeliac plexus. It was first shown that this plexus can organize inhibition of duodenal motility after an increase in gastric internal pressure [1], the so-called gastroduodenal inhibitory reflex (GIR). The ability of the coeliac plexus to organize such a reflex lies in the fact that this plexus has complex integrative properties and should be considered as a true integrative nervous centre [2–4]. Indeed, the motor neurones located in this plexus receive the projections of sensitive fibres originating from the viscera, which form local reflex loops regulating the duodenal motility [5–7]. The studies by our team devoted to the organization of GIR by the coeliac plexus rapidly revealed striking properties of this reflex. We first showed that the reflex was organized without action potentials along the sensitive and motor fibres [8]. We then established that the neurotransmitter released by the sensitive fibres to activate the ganglionic neurones is gaseous: nitric oxide (NO) [9]. Finally we have elucidated some steps of the atypical mechanism conducting without action potentials the excitation along the peripheral nerve fibres [10]. The existence of this mechanism represents a striking new result in the field of neuronal functioning. A historical survey of these studies thus shows that research in biological gastroenterology has led to important advances in fundamental neuroscience.

Characteristics of the gastroduodenal inhibitory reflex organised by the coeliac plexus

The coeliac plexus belongs to the sympathetic prevertebral ganglia. Anatomically it represents the relay between the preganglionic fibres located in the thoracic splanchnic nerves and the ganglionic neurones innervating the viscera. Physiologically it is a true peripheral nervous centre involved in the regulation of various functions such as gut motility, blood pressure, secretion and absorption [4,11]. This plexus innervates in particular the stomach and the duodenum and organizes the GIR.

We have studied this reflex in the rabbit on an in vitro integrated physiological model. The preparation consisted of the stomach and duodenum connected to the coeliac plexus. It was placed in an organ bath with three adjacent compartments: the first contained the coeliac plexus, the second the viscera (stomach and duodenum) and the third the peripheral nerve fibres connecting the celiac plexus to the viscera (Fig. 1A). All these compartments could be superfused independently with drugs. Under these experimental conditions, the duodenum showed phasic contractions occurring with a frequency of 10–20 per min and an amplitude of 250–650 Pa. Gastric distension triggered an inhibition of duodenal contractions up to 80%. This phenomenon occurred with a latency varying from 1 to 10 min and lasted from 5 to 45 min. It characterizes the GIR (Fig. 1B). This reflex was organized by the coeliac plexus since it was abolished after section of the nerve fibres connecting this plexus to the viscera [1,8].

Within the coeliac plexus, it was likely that gastric sensitive fibres synaptically activated the ganglionic neurones, which innervate the duodenum. A synaptic activation is due to the release by one neurone (the presynaptic neurone) of a neurotransmitter, which excites a second neurone (the post synaptic neurone). The release of the neurotransmitter is triggered by excitation of the presynaptic neurone and involves an entry of calcium ions from the extracellular compartment. This mechanism underlies the functioning of all neuronal networks. It is possible to block the release of a neurotransmitter with specific solutions (low calcium-high magnesium solutions), which prevent the entry of calcium. When the coeliac plexus was specifically superfused with such a solution, the GIR was blocked [8]. This result confirms that a synaptic mechanism is required within this plexus during GIR organisation: gastric mechanosensitive nerve fibres activate ganglionic neurones inhibiting the duodenum.

The only mechanism known so far to conduct excitation along the nerve fibres was the action potential. Under the
basal conditions, the inside of the neurone possesses a global negative electrical charge, its resting membrane potential being around -50 mV. During the action potential, the neurone depolarizes rapidly and its inside reaches a positive potential during a few milliseconds. This rapid depolarisation of the neuron is due to the entry through specific channels of extracellular ions with a positive electric charge such as sodium or calcium. Depending upon the type of nerve fibres involved, this excitation is conducted at a speed of 0.1—120 m/sec. The GIR was unaffected after selective superfusion of the nerve fibres connecting the coeliac plexus with tetrodotoxin, a blocker of the sodium channels (Fig. 2) [8]. This blocker prevents sodium contained in the extracellular compartment from entering the neurone thus blocking the sodium action potentials. When the nerve fibres were superfused with a modified Krebs solution where sodium was omitted GIR was not affected. These results indicate that sodium action potentials are not involved in GIR organisation. GIR was also not affected by superfusion of the nerve fibres with a blocker of the calcium channels such as cadmium chloride or with a calcium free solution [8]. This shows that calcium action potentials are not involved in GIR organisation. However, sodium and calcium action potentials being the only ones known in mammals, we concluded that GIR was organised without action potentials along the sensitive or motor fibres. This was a striking result which gave grounds for rethinking one of the central dogmas in neuroscience according to which excitation is only conducted along nerves by an electrical phenomenon, the action potential.

**Neurotransmitter involved in synaptic transmission within the coeliac plexus during gastroduodenal inhibitory reflex**

The determination of the nature of the neurotransmitter involved in GIR organization within the coeliac plexus was the next step of our studies. Considering the atypical properties of this reflex, which is organised without action potentials along the nerve fibres, the involvement of a classical neurotransmitter was unlikely. We decided to look for the involvement of an atypical neurotransmitter and thought that NO, a gaseous neurotransmitter, could be a good candidate. NO is produced from its precursor, the amino-acid L-Arginine, following the action of enzymes called NO synthases. When the coeliac plexus was selectively superfused with Nitro-L-Arginine (L-NOARG), an inhibitor of NO synthases, GIR was inhibited (Fig. 3). GIR was also inhibi-
superfused with ODQ, a selective inhibitor of NO-activated triphosphate (GTP), \[16,17\]. When the coeliac plexus was the GIR. From Quinson et al., 1999, with permission.

Nitric oxide is the neurotransmitter released by mechanosensitive afferences in the coeliac plexus. A and B were obtained from the same preparation. A: gastric distension triggered a gastroduodenal inhibitory reflex (GIR) characterized by the long-lasting (22 min) decrease in the amplitude of duodenal contractions. B: When the coeliac plexus was superfused with L-NOARG (10^{-5} M), the same gastric distension failed to produce the GIR. From Quinson et al., 1999, with permission.

Figure 3

Conduction of excitation without action potentials along the nerve fibres during gastroduodenal inhibitory reflex

Discovering the nature of the molecular mechanism conducting during GIR excitation without action potentials along the nerve fibres was the next challenge for our team. The velocity of this conduction, around 1 cm per minute, is intermediate between the fastest axonal flow of molecules described along the nerve fibres, 40 cm per day \([21]\) and the slowest action potentials (0.1 m per second). During the GIR, the excitation is conducted over 5 cm along the sensitive and motor nerve fibres, which implies the involvement of an active mechanism. So we looked for a possible role of molecules located in the neuronal membrane.

This membrane contains specialized areas called lipid rafts, enriched in cholesterol and particular proteins involved in cell signalling such as receptors, second messenger precursors etc \([22–24]\). We first characterized the presence of lipid rafts in the peripheral nerves organizing the GIR by various biochemical techniques: protein and cholesterol assay, mass spectrometry, immunological detection of lipids and proteins \([10]\). These rafts are known to be enriched in a particular lipid family called sphingolipids. These lipids play a role in the structure of the membrane but are also second messengers involved in cell signalling after activation of specific receptors located on the neuronal membrane.

We then studied the membrane lipids contained in the nerve fibres by gas liquid chromatography techniques. During GIR we showed an increase of ceramide, a sphingolipid, whereas the other lipids such as phospholipids, cholesterol, diacylglycerol or triglycerides remained unchanged (Fig. 4A). The GIR and the ceramide production were abolished after superfusion of the nerve fibres with a selective inhibitor of sphingomyelinase, the enzyme involved in ceramide production. In the absence of gastric distension, superfusion of the nerve fibres with a bacterial sphingomyelinase triggered inhibition of duodenal motility mimicking the GIR and an increase of ceramide content in the nerve fibres. A ceramide analogue, C_{23}-ceramide, superfused either in the ganglionic or the nerve fibre compartment, induced inhibition of the duodenal contraction mimicking the GIR. Interestingly, inhibition of duodenal contraction induced by C_{23}-ceramide at the ganglionic level was blocked when the sphingomyelinase activity was blocked downstream at the nerve fibres level. Taken

\[ \text{guyanate cyclase, the GIR was blocked [9]. This result indicates that during the GIR, NO released within the celiac plexus by gastric sensitive fibres activates a c-GMP production, probably in the cell bodies of the ganglionic neurones.} \]

The involvement of a gaseous neurotransmitter in GIR organization was another feature confirming the atypical properties of this reflex. This result was also of importance in the field of neuroscience. When we published our study at the end of the nineties, a neurotransmitter role of NO in a nervous structure had never been described. The only known role for NO was a modulatory action of neurotransmitters released at neuro-neural synapses in central and peripheral nervous structures \([18–20]\).
Figure 4  Conduction of neuronal excitation along the afferent and efferent nerve fibres involves the recurrent activation of the second messenger cascade: ceramide, calcium, nitric oxide (NO), c-GMP. A: lipid content of the nerve trunks before and during gastroduodenal inhibitory reflex (GIR). During the GIR, only ceramide content increased in the nerve fibres connecting the coeliac plexus to the viscera. PL, phospholipids; SM, sphingomyelin; Cer, ceramide; Chol, cholesterol; DAG, diacylglycerols; TG, triglycerides. Ctrl, (white bars); GIR after GD, (dark bars). The GIR is blocked by superfusion of the nerve trunks with 13 μM BAPTA A/M (B), 1 mM L-NAME (C), 2 μM ODQ (D). Results are given as the percentage of control ± SEM. From Fasano et al., 2007.

Figure 5  Model of neuronal conduction without action potentials. Activation of neutral sphingomyelinase triggers ceramide production in lipid rafts then the release of calcium from intracellular stores, which activates the NO-cGMP pathway. This pathway activates downstream sphingomyelinase in neighbouring rafts, which ensures the propagation of the excitation. L-Arg: L-arginine, NOS: NO synthase, GC: guanylate cyclase, nSmase: neutral sphingomyelinase. From Fasano et al., 2007.
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together, all these results demonstrate that the conduction of excitation without action potentials requires the recurrent production of ceramide along the nerve fibres.

Ceramide is a highly hydrophobic second messenger that remains localized in the membrane lipid rafts [25]. So we formed the hypothesis of the involvement of hydrophilic second messengers localized in the cytoplasm that could propagate the excitation from rafts to rafts. Ceramide is well known to modulate calcium homeostasis [26,27] but also to activate NO-synthases, [28—30] so we looked for the involvement of calcium and NO in the recurrent production of ceramide. We had previously demonstrated that extracellular calcium was not involved in the GIR [8]. However, when the intracellular calcium concentration was lowered by superfusing the nerve fibres with BAPTA/AM, a calcium chelator which crosses the membrane, the GIR was inhibited (Fig. 4B). This result indicates the involvement of an intracellular calcium release in the conduction of excitation without action potentials.

The neuronal NO synthase being calcium dependent, we formed the hypothesis that the increase in intracellular calcium concentration could have led to NO production. Indeed, when the nerve fibres were superfused with Nitro-L-Arginine methyl ester (L-NAME), an inhibitor of NO synthases, the GIR was inhibited (Fig. 4C). In addition, in the absence of gastric distension, superfusion of the nerve fibres with DEA/NO, a NO donor, triggered inhibition of duodenal motility mimicking the GIR. These results show that the activation of the NO synthase and then the production of NO are required for the neuronal conduction of excitation without action potentials. In addition, the GIR was inhibited after superfusion of the nerve fibres with ODQ (Fig. 4D), an inhibitor of the guanylate cyclase which is the enzyme producing the second messenger c-GMP. This shows that the NO produced had activated the synthesis of c-GMP. Moreover C2-ceramide-induced inhibition of the duodenal contractions was abolished when calcium, NO or c-GMP production was blocked.

Taken together, all these results demonstrate that the mechanism of conduction of excitation without action potentials requires along the nerve fibres the production of ceramide in membrane lipid rafts and the activation in cascade of the following second messenger sequence: intracellular calcium, NO, c-GMP [10]. This sequence of second messengers is activated in cascade from rafts to rafts to ensure the conduction of excitation over a long distance (Fig. 5).

**Conclusion**

An overview of the GIR organisation by the coeliac plexus is presented in Fig. 6. The gastric distension activates sensitive fibres which starts the neuronal conduction of excitation without action potentials involving from raft to raft the activation of the following second messenger sequence: ceramide — intracellular calcium — NO — c-GMP. In the coeliac plexus, the entrance of extracellular calcium at the terminals of the sensitive fibres triggers the activation of the NO-synthase and then production of NO, which acts as a neurotransmitter to activate the ganglionic neurones innervating the duodenum. In these neurones, NO activates the production of c-GMP, which triggers in turn the activation of the second messenger sequence to conduct the excitation along the motor nerve fibres.

The classical functioning of neuronal networks involves conduction of excitation based on the propagation of action potentials along the nerve fibres and the activation of membrane receptors due to the release of neurotransmitters. During the GIR, the functioning of the network involved in its organization is fundamentally different. Indeed, the same molecule, NO, is involved in both the conduction of excitation and in the communication between neurones. In addition, this conduction occurs without action potentials and gives grounds for rethinking one of the central dogmas.
in neuroscience according to which excitation is only conducted along nerve fibres by an electrical phenomenon, the action potential.

So, the analysis of the mechanisms involved in the nervous control of gastric emptying by peripheral neuronal networks has led to the discovery of new neuronal properties. These striking results show how studies in biological gastroenterology can have a strong impact in the field of fundamental neurosciences.

Conflict of interest

None.

References


