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Neuroplasticity and neuroprotection in enteric neurons: Role of epithelial cells

Raphaël Moriez [a,b,c], Hind Abdo [a,b,c], Tanguy Chaumette [a,b,c], Magali Faure [d], Bernard Lardeux [a,b,c], Michel Neunlist [a,b,c,*]

[a] INSERM, U913, 1, Place Alexis Ricordeau – 3HNB, Nantes F-44093, France
[b] Université de Nantes, Faculté de Médecine, Nantes F-44093, France
[c] CHU Nantes, Hôtel Dieu, Institut des Maladies de l'Appareil Digestif, Nantes F-44093, France
[d] Nestlé Research Center, CH-100 Lausanne 26, Switzerland

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ABSTRACT

Neurons of enteric nervous system (ENS) regulate intestinal epithelial cells (IEC) functions but whether IEC can impact upon the neurochemical coding and survival of enteric neurons remain unknown. Neuro-epithelial interactions were studied using a coculture model composed of IEC lines and primary culture of rat ENS or human neuroblastoma cells (SH-SY5Y). Neurochemical coding of enteric neurons was analysed by immunohistochemistry and quantitative PCR. Neuroprotective effects of IEC were tested by measuring neuron specific enolase (NSE) release or cell permeability to 7-amino-actinomycin D (7-AAD). Following coculture with IEC, the percentage of VIP-immunoreactive (IR) neurons but not NOS-IR and VIP mRNA expression were significantly increased. IEC significantly reduced dopamine-induced NSE release and 7-AAD permeability in culture of ENS and SH-SY5Y, respectively. Finally, we showed that NGF had neuroprotective effects but reduced VIP expression in enteric neurons. In conclusion, our study identified a novel role for IEC in the regulation of enteric neuronal properties.

Introduction

The enteric nervous system (ENS) is an integrative neuronal network organized along the gut which regulates gastrointestinal functions. Part of this regulation occurs via the liberation of mediators to the different target cells innervated by the enteric neurons such as intestinal epithelial cells (IEC). In particular, recent studies have shown that enteric neurons control intestinal epithelial barrier (IEB) function such as electrolyte secretion, barrier resistance and intestinal cells proliferation [1]. Conversely, enteric neurons are also under the control of its cellular microenvironment and in this context the IEB plays a crucial role. In particular, it is now well admitted that the IEB, and in particular enterochromaffin cells are able to transduce luminal signal to the enteric neurons via their secretion of serotonin and to activate enteric reflexes controlling intestinal peristaltism or mucosal secretion [2,3]. Also, mucosal application of short chain fatty acids or acidity induced the activation of myenteric neurons which depends on the presence of the mucosa [4]. Mechanical deformation of the mucosa is also able to activate myenteric neurons [5]. Further, supporting this regulation of neuronal excitability by the mucosa is the observation that *ex vivo* mucosal removal leads to a reduced neuronal excitability [6]. Besides these short term effects, emerging evidence suggest that the IEB can also impact over long term upon neuronal functions. It has been shown that erbB2 signalling in the colonic epithelia is required for the postnatal survival of enteric neurons [7]. In addition, Caco-2 IEC lines have been shown to favour neurite outgrowth in PC12 cells [8]. However, whether IEC can exert neuroprotective effects is currently unknown. In addition, a recent study has shown that IEC can impact on the ENS by regulating neuronal synthesis of chemokines. In particular, under basal condition, IEC reduced neuronal mRNA expression of IL-8 and MIP-1β in neuronal cell lines [9]. Following stimulation of the IEC by TNF-α/IFN-γ, IEC directly increased neuronal production or mRNA expression of cytokines [9]. However, besides regulation of neuronal production of chemokines, it remains currently unknown whether IEC can also regulate the neuronal expression of neuromediators. Therefore, using coculture of IEC lines and primary culture of rat ENS or neuroblastoma cell lines, we aimed at characterizing whether IEC can modulate neuromediators expression and exert neuroprotective effects.

Materials and methods

Cell culture. Primary cultures of rat ENS were obtained as previously described [10]. Human neuronal-like SH-SY5Y cells were cultured as previously described [11]. Caco-2, T84, IEC-6 (ATCC)

Abbreviations: ENS, enteric nervous system; IEB, intestinal epithelial barrier; IEC, intestinal epithelial cell; NGF, nerve growth factor; nNOS, neuronal nitric oxyde synthase; NSE, neuron specific enolase; VIP, vasoactive intestinal peptide.

* Corresponding author. Address: INSERM, U913, 1, Place Alexis Ricordeau – 3HNB, Nantes F-44093, France. Fax: +33 2 40 08 75 06.
E-mail address: michel.neunlist@univ-nantes.fr (M. Neunlist).

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were seeded in 12-well Transwell® filters (Corning, NY, USA) at a density of 2×10^5 cells/insert and cultured for 13 days to obtain confluence as previously described [12]. All cells were maintained in 95% air, 5% CO_2 at 37 °C.

Table 1
List of primary antibodies.

Raised against	Host species	Source or reference	Dilution
Hu proteins (HuC/D)	Mouse	Molecular Probes, OR, USA	1:200
VIP	Mouse	US biological, MA, USA	1:800
nNOS	Rabbit	Alexis Biochemicals, CA, USA	1:2000
P75	Rabbit	MC192 (gift from Dr. E.G. Johnson) [13]	1:3500
trkA	Rabbit	Kindly provided by Dr. L. Reichard [14]	1:5000
Active caspase-3	Rabbit	Sigma, Saint-Quentin Fallavier, FR	1:500

Table 2
List of secondary antibodies.

Antibody	Source or reference	Dilution
FluoroProbes488 donkey anti-mouse	Interchim, Montluçon, FR	1:200
Cy3-conjugated donkey anti-mouse	Jackson Immunoresearch, BA, USA	1:500
Cy5-conjugated goat anti-rabbit	Jackson Immunoresearch, BA, USA	1:500
Cy3-conjugated donkey anti-rabbit	Jackson Immunoresearch, BA, USA	1:500

Neuro-epithelial coculture model. Forty-eight hours prior to coculture with primary ENS or SH-SY5Y, IEC media were replaced by primary ENS or SH-SY5Y media, respectively. Coculture were performed by maintaining primary ENS or SH-SY5Y cells in presence or absence of IEC for 3 days (neurochemical coding experiments) or 1 day (neuroprotection experiments).

Oxidative stress. Oxidative stress was induced during 24 h with dopamine. Preliminary experiments show that neurotoxic effect in SH-SY5Y cells reaching a maximum at a concentration of dopamine of 1.2 mM (data not shown). This dose was chosen for the rest of the study.

Immunohistochemical analysis. At the end of the experiments, primary culture ENS was processed for immunohistochemical studies as previously described [10]. Primary culture of ENS was incubated in primary antibodies (Table 1) for 90 min. Following 3×10 min washes with PBS, they were incubated for 30 min with secondary antibodies (Table 2). In a second step, all neurons were stained with antibodies against the neuronal marker HuC/D. The number of VIP-, nNOS-, active caspase-3- and Hu-IR cells was counted in at least 20 ganglia per condition. The data were expressed as a percentage normalized to the total Hu-IR neurons.

Quantitative PCR analysis. Total RNA isolation and PCR were performed based on previously described methods [10]. The following primers were used: S6 forward: 5′-CCA AGC TTA TTC AGC GTC TTG TTA ACT CC-3′, S6 reverse: 5′-CCC TCG AGT CCT TCA TTC TC TTG

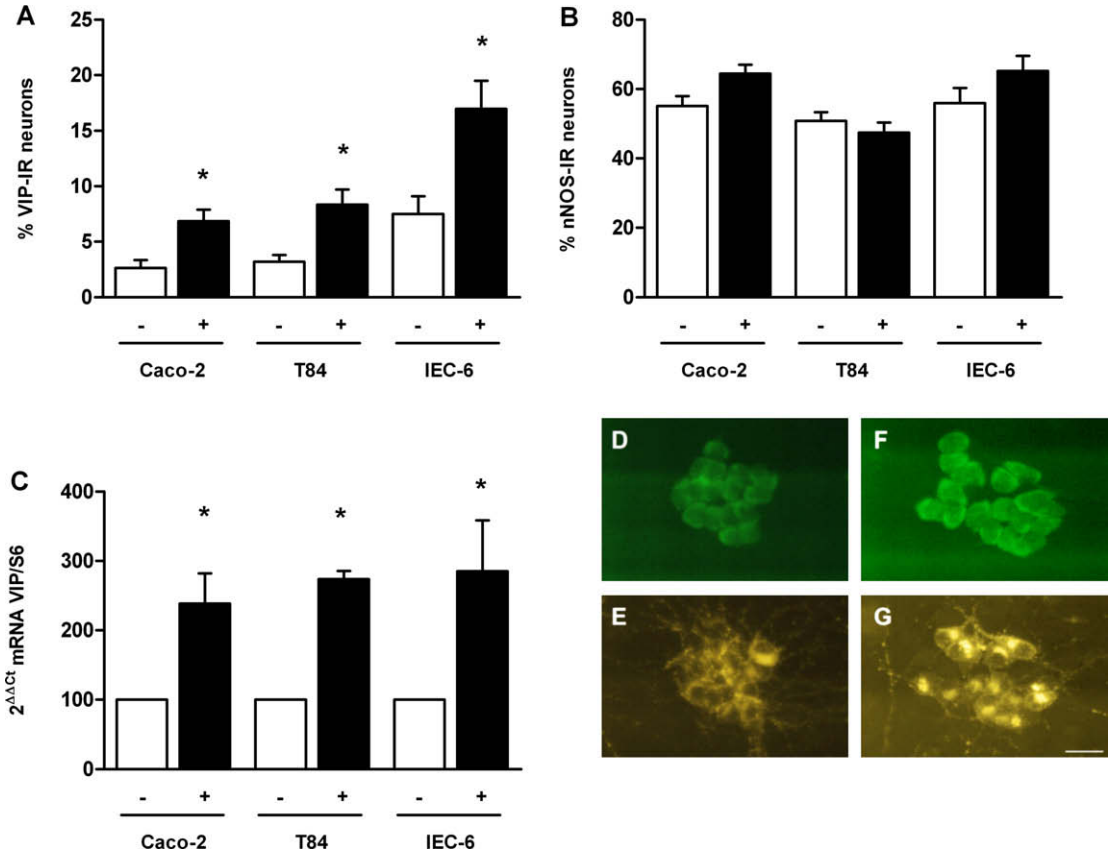

Fig. 1. Intestinal epithelial cells (IEC) modulate the neurochemical coding of the ENS. Primary cultures of ENS were cultured with or without IEC (Caco-2, T84 or IEC-6 cells) for 72 h. Culturing IEC with ENS induced a significant increase in the proportion of VIP-immunoreactive (IR) (A) but not nNOS-IR neurons (B) ($n = 6$, paired t test, $^*p < 0.05$ compared to control). VIP mRNA levels in ENS cultured with IEC were significantly larger than in primary ENS alone (C) ($n = 6$, paired t test, $^*p < 0.05$ compared to control). Values represent means ± SEM. Double immunohistochemical labelling of primary ENS cocultured with or without IEC. Images showed that a larger number of neurons (identified with Hu) were VIP-IR in presence of IEC (F,G, respectively) as compared to control (D,E, respectively). Scale bar = 20 μm.