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Signal in Rats: Influence of Epidermal Growth Factor (Egf)

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Summary

Somatostatin (SRIF) is widely distributed throughout the body. This hormone is an important regulator of the endocrine system through its interactions with pituitary growth hormone, thyroid stimulating hormone, and most hormones of the gastrointestinal tract.

Somatostatin is present in the central nervous system (CNS) where it affects rates of neurotransmission and is also reported to be active in the intestinal tract with evidence that stressed rats present a significant decrease in antral somatostatin-like immunoreactivity (SLI). Analysis of SRIF have been carried out mainly by means of radioimmunoassay methods.

Here we propose the use of an electrochemical method such as differential pulse voltammetry associated with treated carbon fibre micro electrodes (DPV-mCFE) to ease the analysis of such peptidergic electroactive hormone in gastric tissue as well as it has been demonstrated earlier in the rat striatum.

Keywords: Somatostatin (SRIF); Rat stomach; Differential pulse voltammetry (dpv) carbon fibre micro-electrode (mCFE)

Introduction

Somatostatin (also known as growth hormone inhibiting hormone (GHIH) or somatotropin release-inhibiting factor (SRIF) is widely distributed throughout the body. This hormone is an important regulator of the endocrine system through its interactions with pituitary growth hormone, thyroid stimulating hormone, and most hormones of the gastrointestinal tract [1].

Somatostatin affects rates of neurotransmission in the central nervous system (CNS) and proliferation of both normal and tumorigenic cells [2]. However, in a number of cancerous cell lines, somatostatin has also been found to inhibit EGF-induced cell proliferation [3,4].

Prolonged infusion of somatostatin has been found to inhibit gastric mucosal cell division, in ad¬dition, administration of somatostatin together with gastrin has been shown to diminish the gastrin-mediated stimulation of cell prolifer¬ation in the gastric mucosa, indicating an interaction also between the two hormones [5].

It has been shown that in rats submitted to stress i.e. by water immersion [6] the ulcer index of gastric mucosa is significantly higher than that in control rats. In particular, the stressed rats presented a significant decrease in antral somatostatin like immunoreactivity (SLI). It is also known that treatment of stressed rats with epidermal growth factor (EGF), results in an ulcer index significantly lower than that in stressed rats treated with vehicle. This indicates that EGF exerts a cyto-protective activity on gastric mucosa and taken together with the evidence that EGF treatment determines levels of antral SLI significantly higher than that of control rats, proposes a role for EGF in preventing stress ulcer formation. Furthermore, it suggests that endogenous SLI may account, at least in part, for its anti-ulcer activity.

Analysis of SRIF has been carried out mainly by means of radioimmunoassay methods [7,8] i.e. measured in plasma by RIA after ethanol extraction [9]. Here we present an electrochemical method i.e. Differential pulse voltammetry (DPV) to analyze the feasibility of monitoring SRIF in rat gastric preparation. Up to now, DPV associated with specifically electrically pretreated carbon fiber microelectrodes (mCFE), has been mainly utilized to develop sensitive assays for amine neurotransmitters and metabolites in discrete brain regions of rodents [10]. In addition, neuropeptides containing tyrosine, tryptophan and/or cysteine appear to be electrochemically active between +600 and +900mV *in vitro* in a buffered solution at pH 7.4 when analyzed with DPV-mCFE. In particular, we have observed that in vivo in rat striatum, the signal monitored at approx +800mV and called Peak 5 corresponds to the oxidation of somatostatin (which in

fact oxidizes *in vitro* at approx +800mV), or a structurally related peptide [11].

Methods and Results

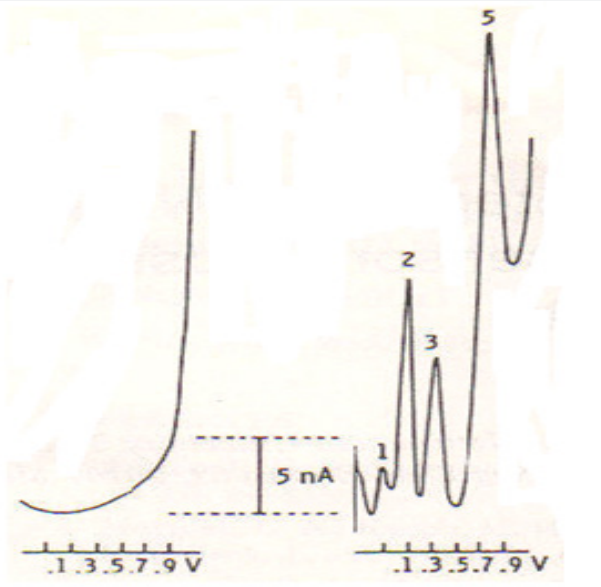


Figure 1: *In vitro* DPV-mCFE scans obtained in PBS, 0.1M; pH 7.4 (LEFT) or in a PBS solution containing a mixture of ascorbic acid (AA) 5mM; DOPAC, 50MM; 5HIAA, 25MM; and somatostatin, 1mM; in PBS, 0.1M; pH 7.4. Peak 1, AA at -50mV; Peak 2, DOPAC at +100mV; Peak 3, 5HIAA at +300mV; and Peak 5, SRIF at +800mV [modified from Crespi 1991 with permission].

The electrochemical activity of SRIF dissolved in saline (vehicle, NaCl 0.9%) was determined *in vitro* by the association DPV-mCFE using a Tacussel PRG5 polarograph with the electrochemical electrodes (auxiliary, reference, and working mCFE) suspended in a 500µl solution of the peptide. The auxiliary electrode was a platinum wire; the reference electrode was a micro silver/silver chloride electrode, the mCFE were prepared using a 12µm-diameter carbon fiber (Carbon Lorraine, France) as described [10,12]. Before use each mCFE was electrically treated in PBS (0.1M, pH 7.4) as described [11]. This treatment enables the detection of three separate peaks *in vitro* in a solution of ascorbic acid 5mM; DOPAC, 50µM; and 5HIAA, 25µM, as already demonstrated [10]. Furthermore, it also allows the *in vitro* detection of a further oxidation peak when the DPV recordings were made in the same solution with the addition of SRIF (Figure 1). The scan rate used was 10mV/s from -250 to +950mV at a step size of 50mV. Then a group of adult male rats (220g weight) was sacrificed and the stomach antrum was obtained as described [7].

Each antrum was then dissected, so that one part was incubated during 120min with:

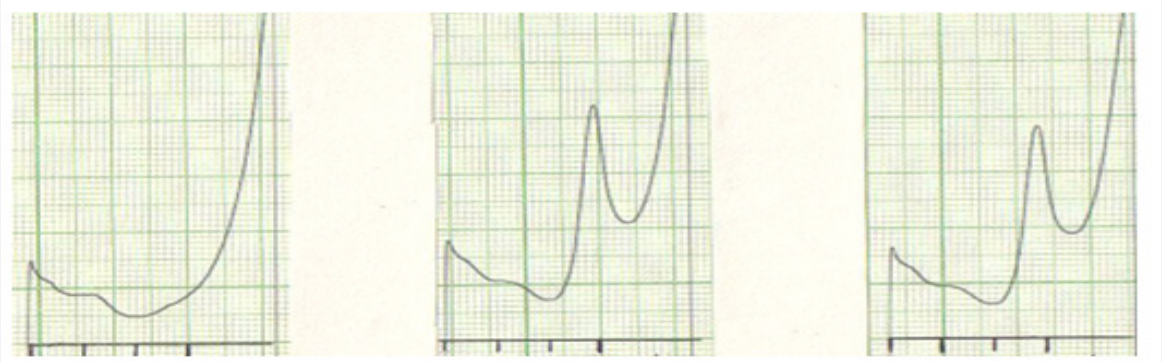
a) Vehicle ([PBS);

b) Antibodies for somatostatin (rabbit polyclonal, IgG antiSRIF AB5494; Millipore (MERCK) or with non specific antibodies as described by [13]

c) Cysteamine 1mM;

d) EGF 1mM.

Successively each gastric tissue was homogenized in PBS at zero degrees (°C), in a ratio of 1:4 weight/volume using a glass-glass homogenizer potter (SAVI Milan, Italy). All the homogenates were centrifuged at 11,000g at 4 °C for 10min. Then each clear supernatant was collected and in the one obtained from the antrum fraction incubated in vehicle, DPV-mCFE measurements were performed showing the presence of 2 oxidation signals. A small oxidation signal at approximately 400/450mV and a taller oxidation signal at approximately 800mV. Both signals were not significantly affected following incubation in aspecific IgG (Figure 2A). In contrast, incubation in specific IgG antiSRIF was followed by a large decrease of the peak recorded at 800mV (Figure 2B). Furthermore, this signal was almost vanished following incubation in cysteamine (Figure 2C). In contrast, incubation in EGF resulted in significant selective increase of the size of the peak monitored at 800mV (Figure 2D). Data are presented as % of control pretreatment values ± SD, *p<0.05, Student T test.



Left: DPV SCANS obtained in the gastric tissue of a single animal to illustrate the effect of incubation with:
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Figure 2A: Aspecific IgG antisera.

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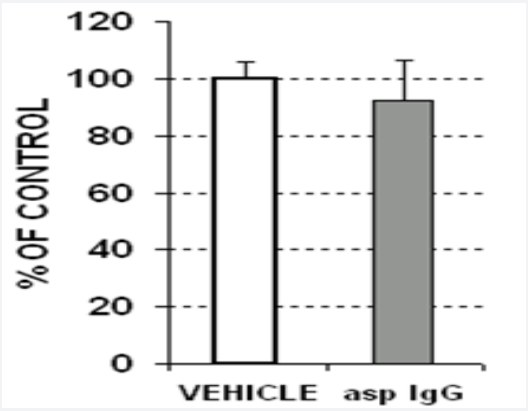

Figure 2B: Aspecific IgG antisera.
Left: DPV SCANS obtained in the gastric tissue of a single animal to illustrate the effect of incubation with:
Right: data obtained in antral preparation from 5 rats, results are expressed in % of control values [size of the peak monitored at 800mV before any treatment], mean ± SD, *p<0.05, Student T test.

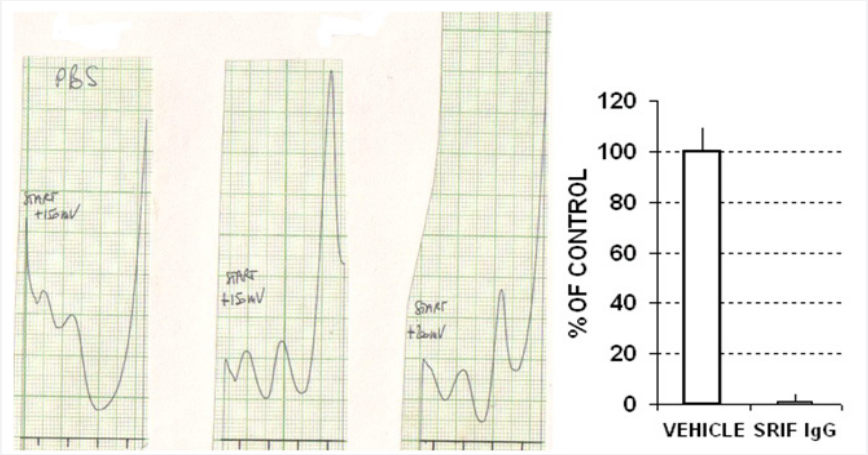

Figure 2B: SRIF IgG.
Left: DPV SCANS obtained in the gastric tissue of a single animal to illustrate the effect of incubation with:
Right: data obtained in antral preparation from 5 rats, results are expressed in % of control values [size of the peak monitored at 800mV before any treatment], mean±SD, *p<0.05, Student T test.
In Figure 2B note the presence of three peaks before treatment, with Peak 3 obtained at +800mV. Following 120min incubation with specific antisera the peak 3 disappeared completely.

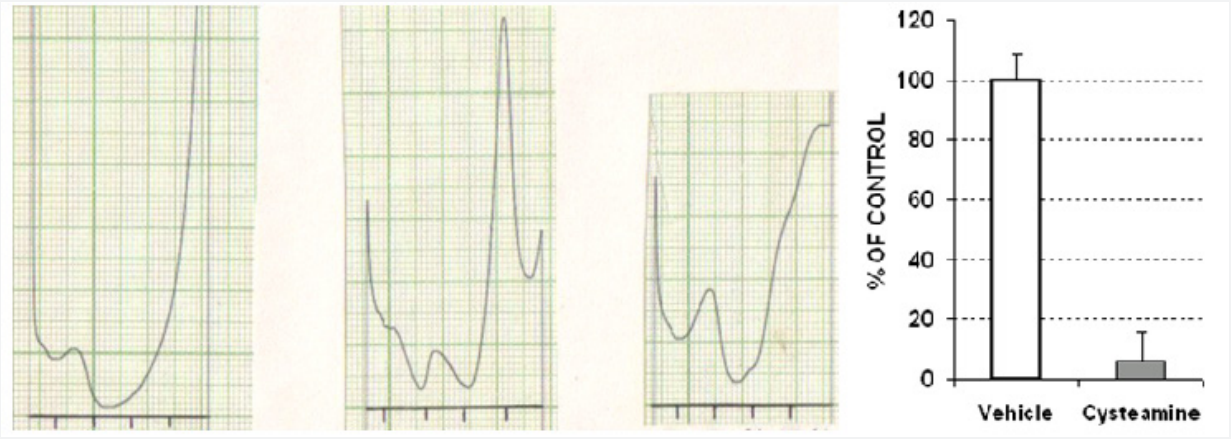

Figure 2C: Cysteamine.

Left: DPV SCANS obtained in the gastric tissue of a single animal to illustrate the effect of incubation with:

Right: data obtained in antral preparation from 5 rats, results are expressed in % of control values [size of the peak monitored at 800mV before any treatment], mean±SD, *p<0.05, Student T test.

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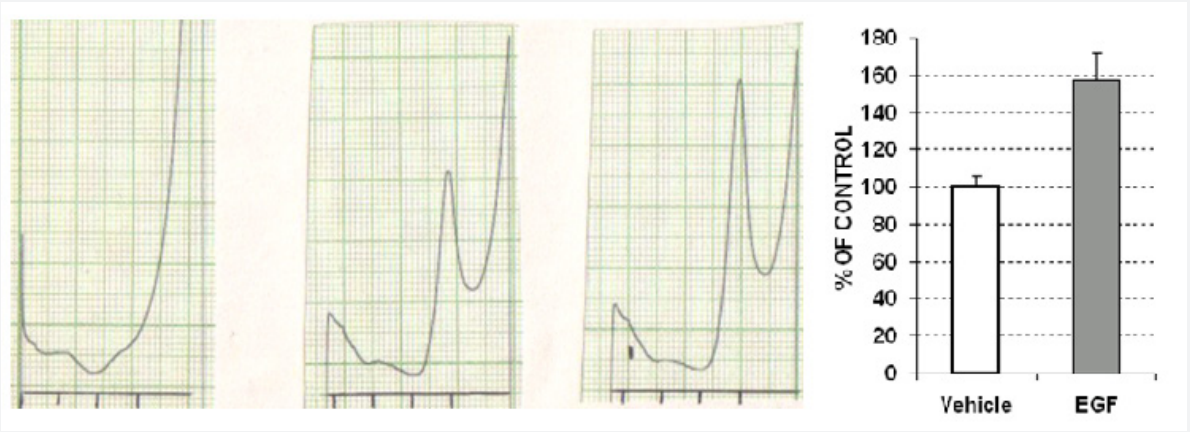

Figure 2D: EGF.

Left: DPV SCANS obtained in the gastric tissue of a single animal to illustrate the effect of incubation with

The size of the peak recorded at +800mV and considered due to the oxidation of SRIF was measured in nano Amperes [nA].

Right, data obtained in antral preparation from 5 rats, results are expressed in % of control values [size of the peak monitored at 800mV before any treatment], mean ± SD, *p<0.05, Student T test.

Discussion

Somatostatin is an important regulator of endocrine and nervous system function. It exerts its biologic actions by binding to specific high affinity receptors on the cell surface. Somatostatin has also been shown in sympathetic nerves, mucosal cells, myenteric nerves of the gastrointestinal tract, salivary glands and in some parafollicular cells of the thyroid [1]. Somatostatin is widely distributed within the CNS and relatively high levels are found in the striatum [14,15].

Cysteamine is a thiol reagent reported to selectively affect brain somatostatin and prolactin [2,8,16,17]. Another *ex vivo* study [18] demonstrated that cysteamine given systemically selectively depletes somatostatin in the rat central nervous system while Beal & Martin [19] found that local cysteamine injection decreased the somatostatin content of striatal slices. Similarly, Kwok et al. [20] have shown that single injection of cysteamine produced a rapid decline in immunoreactive somatostatin (IR-SRIF) levels in the hypothalamus of rat brain.

Accordingly, in our previous work [11] peripheral injection of cysteamine decreased the DPV-mCFE signal recorded in the rat striatum at +800mV and called Peak 5 supporting the view that this peak is due to

a) A peptide and

b) Possibly somatostatin. Local injection into the brain striatum of purified somatostatin antisera, which would be expected to combine with the somatostatin in the extracellular space and therefore prevent its oxidation at the surface of the working electrode, also caused the eventual disappearance of Peak 5 within 120 min. conversely, local injection of control a specific antisera had no significant effect on the size of Peak 5, thus providing the original evidence that SRIF can be the principal component of this DPV-mCFE signal in the striatum of the rat brain.

Somatostatin like immunoreactivity (SRIF-LI) has been also measured in the rat stomach and it was observed that gastric SRIF-LI secretion is significantly stimulated in a dose-dependent manner by treatment with norepinephrine and dopamine [21]. Again, it has been reported that cysteamine acts in the stomach lumen at the cellular level to cause breakdown of preformed somatostatin and/or to acutely reduce its synthesis. [2,8]. Accordingly, the voltammetric data presented here have monitored a clear influence of incubation with cysteamine, resulting in a large reduction of the putative gastric SRIF signal recorded at 800mV. Similarly, incubation with specific antisera depleted this signal in gastric preparation a well as it was described happening in the rat striatum [11].

In other studies, in rats submitted to stress applied by water immersion a significant decrease in antral somatostatin like immunoreactivity ([SLI) was monitored together with the evidence of stress-induced antral ulceration. The injections of pentagastrin and/or EGF resulted in substantial increase in antral content of SLI [7]. This is in accord with our experiments showing that antral incubation with EGF resulted in a significant increase of the electrochemical signal occurring at +800mV in the rat gastric tissue when using DPV-mCFE as it was done in brain tissue [11].

Thus this data support the direct influence of EGF upon the DPV-mCFE antral signal recorded at +800mV and provide evidence for the chemical nature of this voltammetric peak as related to gastric SRIF oxidation. Furthermore this data is parallel to our earlier voltammetric in vivo observation of direct interaction between cerebral SRIF and another growth factor such as growth hormone (GH) [23]. In addition, in this cited work a direct catecholaminergic influence upon the SRIF peak was observed in vivo as it was already described *in vitro* [21].

All together the presented voltammetric data support the literature description of influence of cysteamine, EGF, specific

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SRIF antisera and confirm the chemical nature of the gastric signal recorded at 800mV with DPV-mCFE as corresponding to oxidation of gastric SRIF. Up to now analysis of SRIF have been carried out mainly by means of radioimmunoassay methods [2,7,8] i.e. measured in plasma by RIA after ethanol extraction [9]. The electrochemical method DPV-mCFE proposed here for detection of SRIF presents various advantages i.e. lack of the need for excessive sample preparation; selectivity due to electrical signals at characteristic formal potentials, which can enable detection of multiple analytes without separation steps and the ability to analyze within various biological matrices *in vitro* [i.e. serum, cell culture media] as well as *in vivo*. Electrochemical methods also have reasonably fast sampling times, can offer valuable insights on the metabolic outcome of the drug at particular dosage levels inside the body, and allow for investigations of the interaction of drugs with living cells.

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