Antisecretory Factor

1.Introduction

The basis of the development of these products goes back to the first half of the 1980's. At that time a new endogenous factor (AF) was discovered by a research group at the Institute of Microbiology of the University of Goteborg (Sweden).
This factor was initially demonstrated in the rat (Lonnroth and Lange, 1984a) and later in the pig (Lonnroth and Lange, 1984b). It is induced by *vibrio cholerae* infection, in particular by Cholera Toxin (CT) action in the intestine. Small intestine mucosa and pituitary extracts from infected animals could block intestinal water and electrolyte hypersecretion in an experimental model of hypersecretion in the rat. This model is based on the evaluation of the amount of water secreted in un given time in an isolated small intestine loop ("rat ligated loop" : Lange and Lonnroth, 2001).
AF factor was then isolated and studied. It is an acidic protein whose molecular weight was initially evaluated as 60000 Daltons (Lonnroth et al., 1988).
Later, AF has been cloned and its molecular weight finally determined as 41000 Daltons. The protein has 382 amino acids (Johansson et al., 1995). Subsequently, a 7 amino acids sequence between amino acid 36 and 42 , starting from the N terminal, has been recognized as the one active as antisecretory in the small intestine (Johansson et al., 1997; Lange and Lonnroth, 2001).
In the following, when it is mentioned *active AF*, protein AF exposing that sequence is meant.
The mechanism of the protein antisecretory action is the block of anionic channels and of pores for water molecule permeation (e.g. members of the "aquaporins" family). These are active in inflammatory events and in infections such as that due to cholera toxin. In this case, the cellular cyclic AMP concentration increase in epithelial cells of intestinal mucosa, due to the toxin action, results in pore activation with subsequent water and electrolytes release (for a review: Laohachai et al., 2003).
These "pores" regulate, in practically all body cells, cellular volume when it is altered by extracellular hypo- o hyper-osmosis. In the first instance the cell volume increase is balanced by what is called a Regulatory Volume Decrease (RVD), in the second one there is a Regulatory Volume Increase (RVI). In both cases anionic channels or pores involved in water molecule passage enter into play (Rapallino and Cupello, 2001; Sardini et al., 2003; Darby et al., 2003; Srinivas et al., 2004; Kida et al., 2005; Do et al., 2006; Li and Olson, 2008).

Since the early studies about AF the important role of the pituitary gland in its synthesis and activation has been elucidated (Lonnroth and Lange 1984a, 1984b, 1987). Later, active AF

presence has been demonstrated, after cholera infection, in important biological fluids, such as bile and milk in the rat (Lange and Lonnroth, 1986). Obviously, in non mammals AF cannot be in milk. Thus, in evolution this could not be a way of defense for the offsprings from hypersecretion due to infections. It is thus very interesting the finding by Stefan Lange's group that in the hen egg AF is concentrated in the yolk (Lange et al., 1994).

In addition, Lonnroth e Lange nel 1987 observed that pituitary AF synthesis was stimulated by the induction of hypersecretion in the intestine by peroral 500 mg amounts of mannose, sorbitol or amino acids (Lonnroth and Lange. 1987). Since all these treatments can cause hyperosmolarity in the intestine, the rationale was that if one induces AF synthesis *via non pathological means* the body could benefit from AF production/activation. This can protect from inflammatory pathologies, as demonstrated by several clinical trials, as reported below.

2. Scientific Concept

AF protein presence has been demonstrated in many organs by immunohisto- and cyto-chemical techniques and its messenger RNA visualized by in situ hybridization (Lange et al., 1999; Davidson and Hickey, 2004). In the various organs (among which the gastro-intestinal tract, the respiratory apparatus and the uro-genital tract) the cells which were positive for the protein were epithelial e limphoid ones. In the pituitary, labelled cells were the endocrine ones (in the adeno-hypophysis) and epithelial ones.

The overall idea is that of a protein factor which is present in the various body organs where it controls hypersecretion by blocking cell anion channels involved in cell volume regulation (Rapallino and Cupello, 2001; Rapallino et al., 2003; Sardini et al., 2003).

This factor is initially activated in peripheral organs, particularly in the intestine. Its activation involves exposing an active segment, close to the protein N terminal (Johansson et al., 1997, 2009). Local activation of AF transmits a message via the nervous system (on which the factor acts: Rapallino et al.,1989, 2003; Kim et al., 2005) to the hypothalamus-pituitary axis. When the intestine is primarily challenged by hypersecretion the message is transmitted via the afferent part of the vagus nerve (X cranial nerve). Then, pituitary AF is released in the active form in the portal system at the adeno-hypophysis. In this way, the blood stream brings the active factor to the peripheral organs, where it acts and in turn activates local AF factor (Tateishi et al., 1999; Lange and Lonnroth, 2001).

The reaction described may be triggered also by situations under which the local hypersecretory event is induced by conditions which are not pathological. Among this is the passage through the

intestine of hypertonic media such as relatively concentrated sugar solutions absorbed on substrates like cereal kernels. This condition is created by the ingestion of cereals previously processed by procedures leading to partial starch hydrolysis (malting).

3. Experiments in animal farming (swines and cattle). Current use in animal farming in Sweden and other scandinavian countries of AF inducing diets.

The concept discussed in paragraphs ***1.*** and ***2.*** has been applied to the attempt of inducing lectins (proteins which like AF can bind glycoprotein sugars) in piglets after weaning by cereals treated in a way to induce controlled non pathological intestinal secretion. Such a food resulted in less diarrhoea and lower mortality rate in the animals. Besides, the piglets displayed greater weight increase in the post-weaning period (Goransson et al., 1993). This type of diet has been later applied extensively in farming of swines and bovines in Sweden. In fact, the introduction of the total ban of antibiotic use in animal food in farms from January 1st, 1986 caused serious problems in animal farming due to a great increase of intestinal pathologies in the animals. The introduction of AF inducing cereals in the animal food from the beginning of the 1990's resulted in a great improvement (Lange and Lonnroth, 2001).
Nowadays, as an example, in Sweden 60 % of swines in farms are grown according to the criteria reported above (Dr. J.I. Bruhn, Lantsmannen AS Faktor, Sweden: personal communication).

4. Clinical observations

Starting from this background, let us examine the clinical trials in which endogenous AF activation strategies have been tested. These treatment involved the use in the food of cereals (**SPC-cereals**) specially treated by a particular hydrothermal procedure enriching them in mono- e di-saccarides. These cereals (corresponding to the product described here as **SPC-Flakes**), are able to produce a hypertonic environment in the intestine. By this treatment clinical improvement has been found not only in patients with intestinal hypersecretion because of Cholera Toxin, but also in patients with inflammatory intestinal pathologies (ulcerative colitis and Crohn disease: Bjorck et al., 2000). The treatment was for 4 weeks and resulted in subjective evaluations (blind) of clinical symptoms which were much more positive in patients treated with SPC cereals than in patients eating cereals which had not processed with the special procedure referred to above. The clinical improvement was parallel to an increase of AF plasma levels in the comparison with controls (taking untreated cereals). Of great efficacy in these pathologies was treatment with preformed active AF prepared

from hen egg yolk (**Salovum**), both when it was given alone (Eriksson et al., 2003a) or together with **SPC-Flakes** (Eriksson et al., 2003b).
Similarly, a positive outcome of **Salovum** or **SPC-Flakes** treatment was demonstrated in cases of diarrhoea of endocrine, origin due to neuroendocrine tumors (Laurenius et al., 2003).
In developing countries, diarrhoea in in children is a huge medical problem. In a trial performed in Pakistan, a product analogous to **Salovum** was shown to reduce the severity of diarrhoea in children (Zaman et al., 2007).

Similarly, positive results were obtained for pathologies due to inflammatory processes, such as mastitis in lactating women. Also here, clinical improvements were found together with parallel active AF increases in maternal milk (Svensson et al., 2004). Besides, very positive indications were obtained in clinical trials for pathologies due to biological fluid hypersecretion, like endolymph in the inner ear in Meniere's disease (Hanner et al., 2004, 2010).
It must be underlined that in almost all pathologies studied, in particularly treatment-resistant cases a short initial treatment with **Salovum** , *which contains exogenous active AF factor,* was shown to be rapidly efficacious. Later in the treatment, exogenous AF (**Salovum**) was gradually substituted with SPC-cereals (**SPC-Flakes**), which induce endogenous AF synthesis.

PRODUCTS

SPC-Flakes and their mechanism of action

In the clinical trials mentioned in the previous paragraph the product mainly used was **SPC-Flakes**, where SPC is an acronym for Specially Processed Cereals.
The starting point in the development of this product was what is reported here at the previous paragraph ***1.*** In particular, the demonstration that in the rat the administration of amounts of monosaccarides (mannitol, sorbitol) and amino acids (glycine, alanine) giving hypertonic media in the small intestine resulted in endogenous AF factor activation in the pituitary gland (Lonnroth and Lange, 1987).
Then, hydrothermal procedures were devised by which the starch and proteins of the cereals (mainly oat) were partially hydrolyzed with enrichment of the cereals in mono-, di-saccarides and amino acids. This resulted in endogenous AF system activation when the cereals were ingested and reached the intestine. The mechanism of the effect is the enrichment in monomers and dimers due to the partial hydrolysis of starch and proteins by the hydrothermal pre-treatment of the cereals.

See the following Table:

TABLE*

Content	**Before the process (mg/g $_{cereal}$)**	**After the process (mg/g $_{cereal}$)**
Glucose	0.3-0.4	0.6-5.3
Fructose	0.3-0.4	0.6-3.1
Sucrose	8.4-14.4	36.8-65.7
Maltose	0.0	4.0-9.0
Histidine	0.0	0.06-0.25
Glutamic acid	0.12-0.20	0.42-0.44
Lysine	0.03-0.06	0.15-0.29
Tryptophan	0.09-0.22	0.28-0.45
Isoleucine	0.0	0.05-0.30

* Content in mg of the various substances per gram of cereal before and after the hydrothermal process.

From these figures, one can calculate that in 35 grams of cereal ingested (at the amount per day of 1g cereal per kg body weight divided in two meals, for a 70 kg person, see e.g. Hanner et al., 2010) the hydrothermal procedure results in an increase of around 7 millimoles. Considering that in man the internal volume of duodenum is in the 0.1-0.15 liter range (26 cm of length and a diameter of 2.5-3 cm) there can be an *average osmolarity increase* of around 50 milliosmol. Leaving alone that the effect is certainly *bigger in the layer at direct contact with the intestinal mucosa*.
The hypertonic medium in contact with the intestinal wall determines an osmotic passage of water and electrolytes in the intestinal lumen. As previously discussed, these events send a signal which results in AF system activation, a homeostatic reaction.
As discussed above at paragraph ***3.***, the induction of this sequence of events was exploited in Sweden in the production of animal food (starting from the 1990's). This animal food, based on a

new concept, brought remarkable improvements in bovine and swine farming. In fact, nowadays about 60 % of swines in Swedish farms are fed with this food after weaning (this food is made on the basis of the same concept as SPC-flakes)
Obviously, it is important to underline the various clinical trials which followed (paragraph ***4.***) which demonstrated **SPC-Flakes** efficacy in pathologies due to inflammation in addition to their efficacy in pathological intestinal hypersecretion (Bjorck et al., 2000; Eriksson et al., 2003b; Svensson et al., 2004; Hanner et al., 2004, 2010).

Conclusion **SPC-Flakes** product is able to *induce the endogenous production of active AF factor*. This happens *within a few weeks*. This product in addition to being efficacious in combatting intestinal hypersecretion is therapeutically efficacious in inflammatory pathologies such as mastitis and Meniere's disease.

SALOVUM

SALOVUM is egg yolk obtained from hens fed with **SPC-Flakes.** This treatment enriches the egg's yolk with active AF factor. It must be remembered that the hen egg's yolk is naturally already rich in AF (Lange et al., 1994). Treating hens with **SPC-Flakes** their egg yolk is further enriched in AF.
As a product, **SALOVUM** is a spray-dried powder obtained from the yolks: 20 mg of this product correspond to 1 unit of AF, 1 unit is defined as the amount of AF which reduces by 50 % hypersecretion in the rat intestinal loop model, after Cholera Toxin infection.

In clinical trials, **SALOVUM** has been given at daily amounts of 10 g.
A point to be clarified is how the product maintains its efficacy after oral ingestion.
The reason is that egg yolk contains, at variance from the albumen, a high level of lipids (about 30 % of the weight). Thus, active AF factor is sequestered in inverse micelle where the hydrophobic moiety of lipids is external and the hydrophilic is internal and contacting the AF protein. In this way the active AF factor "survives" un-denatured in the acid pH of the stomach after ingestion. From the stomach it passes still active to the blood. In fact, the protective lipid shield allows it to pass the stomach wall. After all, this is the principle which led to the lab production of liposomes as a means to transport hydrophilic substances across membranes.

The action of *active exogenous AF* administered in this way is particularly rapid. It requires only around 30 minutes to act.

Daily intake of 10 g of **SALOVUM** was tested in patients with diarrhoea due to intestinal surgical resection because of Crohn's disease (Lange et al., 2003; Eriksson et al., 2003b) and in patients with secretory diarrhoea due to neuroendocrine tumors (Laurenius et al., 2003). It has been tested also in Meniere's disease patients (Hanner et al., 2004).

In these trials the product has been given for a few weeks and then it was gradually substituted with SPC-Flakes for the long term treatment.

In other instances, **SALOVUM** has been used alone, such as in ulcerative colitis (Eriksson et al., 2003a). In addition, recently a product analogous to **SALOVUM** has been used successfully in Pakistan in the treatment of diarrhoea in children (Zaman et al., 2007).

In all instances the results were positive.

Conclusion **SALOVUM** delivers *active exogenous AF factor* and works in *acute treatment* of intestinal hypersecretion and other pathologies, among which Meniere's disease.

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