Intestinal maturation induced by spermine in young animals

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Abstract

In suckling rats, it has been established that oral administration of spermine, a dietary polyamine, at appropriate doses, induces all the modifications in the digestive tract that occur at weaning, namely, (a) in the intestine: variations in the specific activities of disaccharidases and peptidases, gene expression, the level of receptors to polymeric immunoglobulins (RPIs), tissue histology, and the intestinal permeability to macromolecules; (b) maturation of the intestinal immunological system, an observation confirmed in mice; (c) an increase in the specific activity of enzymes contained in the pancreas; (d) a change in the growth rate and biochemical properties of the liver, e.g., in the synthesis of RPIs. The mechanism of spermine action is partly understood. In the intestine, two phases of events have been recorded. The first phase consists of the desquamation of the epithelium resulting from an activation of apoptosis, probably induced by the entrance of spermine into the enterocytes. The second phase concerns a hormonal cascade in which adrenocorticotrophic hormone, cytokines, bombesin and corticosterone intervene. A general hypothesis taking into account all the results obtained until now is presented as well as other observations recorded on the effect of exogenous spermine on the intestinal maturation of vertebrates other than the rat.

Keywords: Polyamine; Spermine; Small intestine; Maturation; Rat

1. Introduction

Maturation of the intestine is known to occur in the first few postnatal weeks in many vertebrates. As the objective of the present review is to report the importance of spermine in this phenomenon, we will first give a short description of postnatal intestinal maturation in the animals where the role of this substance is proven, i.e., in the sea bass (Dicentrachus labrax), the mouse and the rat. Thereafter, we will report on the observations concerning the effects of polyamines, especially spermine, on intestinal maturation.

Marine fish larvae undergo major changes in morphology and functionality of their digestive tract during the first 5 weeks of life, i.e., until they have achieved their development. This process has been well described (for example, Henning, 1987; Cahu and Zambonino Infante, 1995). It essentially consists of modifications in digestive enzyme activity.

In the mouse and rat (Henning, 1981), the small intestinal mucosa undergoes many modifications during the second or the third postnatal week. Among others, the large supranuclear vacuoles contained in the enterocytes of the distal immature intestine disappear (Veress and Baintner Jr., 1971), a
significant decrease in the intestinal permeability towards proteins ("gut closure") is observed and some mucosal enzyme specific activities (SAs) are modified. For example, maltase and sucrase SAs increase in the third postnatal week. Lactase which is essential for lactose hydrolysis during the first two postnatal weeks, and lysosomal enzymes, which intervene in the digestion of chyme molecules, lose a part of their activity and concentration. Some of these phenomena are also observed in babies. This suggests that mice and rats could represent good models to understand biochemical and physiological mechanisms taking place during development of the human small intestine, and to understand or correct causes and consequences of different dysfunctions of the intestine.

Luk et al. (1980) and Luk (1990) have shown that ornithine decarboxylase (ODC) SA and polyamine concentrations increase in the intestinal mucosa during weaning in rats. They established that α-difluoromethylornithine (DFMO), an inhibitor of ODC, delays the intestinal maturation process. Therefore, further research to better evaluate the role played by polyamines in the intestine maturation was undertaken.

Previous observations, taking into account that polyamines are substances playing an important role in tissue proliferation and differentiation, led Dufour et al. (1988) to test the effect of dietary polyamines on intestinal maturation. They orally administered spermine and spermidine to rats, which were 7 or 11 days old, at doses from 3 to 8 μmol twice a day for 3 days. They showed that these treatments induce changes in intestinal enzyme activity similar to those occurring during normal maturation. These results were confirmed by Buts et al. (1993), Wild et al. (1993), Harada et al. (1994) and Dorhout et al. (1996). Georges et al. (1990) extended these observations to the ultrastructure of the cell epithelium in rats. Later on, Wéry et al. (1992) and Osman et al. (1998) proved that these spermine treatments induce "gut closure" in these animals. Buts et al. (1993) demonstrated that the concentration of the secretory component of polymeric immunoglobulins was increased in the villus and crypt cells by spermine ingestion. All these observations suggest that oral ingestion of spermine induces complete intestinal maturation in suckling rats and, thus, could play a role in normal intestinal development.

In mice, it was also demonstrated that spermine induces the maturation of the small intestine and the associated immune system (Ter Steege et al., 1997). In the sea bass, data confirm this assertion (Péres et al., 1997). A diet enriched in spermine can accelerate the appearance of the adult enzymatic profile in the intestine and in the pancreas of this fish species.

2. Polyamines in milk

Since intestinal maturation occurs in the rat for doses of spermine equal to or higher than 1 μmol/day, a likely role of dietary polyamines in normal maturation of the intestine was proposed for this animal.

In order to find out whether ingested polyamines can intervene in the normal development of the rat intestine, the concentration of these substances in rat milk and food was estimated. As shown by Pollack et al. (1992) and Romain et al. (1992), the concentration of putrescine and spermine is low (less than 2.5 nmol/ml) and does not vary much during the lactation period, while the spermidine concentration is higher and seems to increase during lactation (from 9 to > 20 nmol/ml). Rat food contains approximately 150-times more putrescine and spermine, and approximately 30-times more spermidine than rat milk. Thus, solid rat food represents a substantial increase in the exogenous contribution of polyamines at weaning in the rat.

Consequently, the polyamines contained in the milk at the end of suckling and in the solid-rat food could play a role in the postnatal maturation of the rat intestine or in maintaining a functional stage in the bowel. This possibility is reinforced by the fact that bacterial flora, which constitute another source of exogenous polyamines, grow with the administration of solid food (Osborne and Seidel, 1990). Therefore, it appears that further investigation on the intestinal maturation induced by spermine is fundamental.

Polyamine concentrations of the milk were also estimated in the cow (Sanguansermsri et al., 1974; Motyl et al., 1995), sow (Kelly et al., 1991; Motyl et
Peulen et al., 1995), goat (Poszaj et al., 1997) and human female (Sanguansermsri et al., 1974; Pollack et al., 1992; Romain et al., 1992; Buts et al., 1995). In general, goat’s colostrum and milk are rich in polyamines, and concentrations are higher than those in milk of the other mammals. Polyamine concentrations vary with the diet, species, time of lactation, total litter weight, offspring number and milking time. Although there are no data demonstrating a role of dietary polyamines in maturation of the digestive tract (except maybe in babies), there is also no data disproving this possibility.

3. Role of dietary spermine in intestinal development

As spermine could play an important role in the intestinal maturation occurring at weaning, it is useful to carefully analyse its morphological and biochemical effects, and its mode of action.

Peulen et al. (1998b, and unpublished results) confirmed, in the rat, that during the first 3 days of spermine treatment (see earlier), the evolution of the following parameters was similar in both natural and in spermine-induced maturation of the intestine: proteins, RNA and DNA concentrations of the intestinal mucosa, SAs of brush border disaccharidases, such as lactase, maltase and sucrase, morphology of small intestine mucosa and ultrastructure of enterocytes, as well as alkaline phosphatase SA, TNF-α plasma concentration and gene expression (cdx-1, cdx-2, L-FABP and APO A4). Consequently, it was proposed that spermine-induced intestinal maturation is identical to the normal intestinal maturation which occurs at weaning.

However, other observations do not agree with this conclusion: this phenomenon did not appear irreversible (Georges et al., 1990), was not maintained for a long period (Deloyer et al., 1996a) and a strong desquamation of the intestinal epithelium (see later) was not observed at weaning.

Indeed, results indicate that an interruption in the spermine treatment for the experimental conditions usually chosen (8 µmol/day, once a day, for 3 days) was followed by an involution of the enterocyte differentiation (Georges et al., 1990), a phenomenon which was never observed after weaning. Nevertheless, irreversibility of normal intestinal maturation has never been proven, maybe because adult rat diets always contain high polyamine levels as compared with rat milk (Pollack et al., 1992; Romain et al., 1992) (see later) and because intestinal microflora synthesise polyamines (Osborne and Seidel, 1990). Thus, it may be suggested that dietary polyamines are furnished at such an amount after weaning that postnatal maturation cannot return to its suckling state.

Therefore, experiments were performed in order to reduce, where possible, all sources providing polyamines to the intestine of adult rats (Deloyer et al., 1996b). By using germ-free animals, the polyamine supply from intestinal microflora was avoided. Dietary polyamines were reduced by giving a specially defined diet to the rats. Endogenous synthesis of polyamines was limited by administration of DFMO and methylglyoxal bis guanylhydrazone (MGBG), an inhibitor of S-adenosylmethionine decarboxylase and of plasma membrane permeability to polyamines.

This treatment allowed Deloyer et al. (1996b) to observe that: (1) rats with intact intestinal microflora had higher SAs of maltase and higher amounts of spermidine and spermine, but lower lactase SA, than pathogen-free animals; (2) a low polyamine diet given to germ-free rats had little effect on the functional variables analysed (sucrase, maltase and lactase SAs) and did not modify the intracellular amounts of polyamines; (3) DFMO and/or MGBG administered to germ-free rats receiving a low polyamine diet induced modifications in most of the variables studied; (4) body weight and wet weight of the proximal and distal intestine decreased, disaccharidase SA decreased and polyamine amounts changed according to the inhibitor used.

As a whole, these results indicate that the supply of polyamines by microflora or by the diet is not essential for the maintenance of intestinal properties and that a restriction of polyamines, both endogenous and exogenous, alters general properties of the organism as well as intestinal functions. Whatever the treatment applied, modifications of variables indicating an “unmaturation” of enterocytes (i.e., a decrease in maltase and sucrase SAs, an increase in lactase SA and a modification in the ultrastructural
aspect of distal enterocytes) were never observed. Consequently, at weaning or just after this period, irreversible modifications of gene expression could occur in the crypt stem cells, probably as a result of a biological clock effect or of the new hormonal status of the animals (see later).

Another experiment, undertaken with other research goals in mind, was performed in which germ-free rats were fed for 2 months with a synthetic diet containing a very low amount of polyamines (Deloyer et al., 1998). The conclusions obtained confirmed the previous ones. They allow us to conclude that the intestine is an organ which is well protected against such experimental conditions, in contrast to other organs.

On the other hand, as postnatal maturation induced by spermine ingestion appears to be reversible, it may be suggested that a spermine treatment lasting more than 3 days could maintain the adult stage of the enterocytes until the time of weaning, and that this stage would be irreversible. This possibility was also investigated (Deloyer et al., unpublished results). Indeed, when rats ingested spermine for more than 3 days (for doses, see earlier), intestinal wet weight was greater compared with the control rats. In the same way, lactase SAs remained lower in the intestine of the spermine-treated rats. In contrast, sucrase and maltase SAs in the jejunum and in the ileum of the spermine-treated rats decreased when the duration of the treatment was longer than 3 days and finally returned to control values after 6 days. Furthermore, histological studies showed that large supranuclear vacuoles progressively disappeared in the ileum of the rats that were ingesting spermine for more than 3 days.

These experiments, along with other data, suggest that the state of differentiation of enterocytes, other than being fixed and irreversible, could be dynamic and could require continuous control by environmental factors.

In conclusion, it appears that dietary spermine can induce all the studied variables characterising postnatal intestinal maturation; thus, it is likely to play a role in normal intestinal maturation although, by itself, it is unable to maintain the stability or irreversibility of intestinal development in the rat. The possibility to induce phenomena associated with normal intestinal development at weaning by giving spermine orally is an important observation, giving researchers an excellent model in order to investigate the development of the intestine.

4. Evolution of different intestinal variables after the ingestion of a single dose of dietary spermine

In order to investigate the role and effects of spermine administered orally to suckling rats, the evolution of intestinal variables after the ingestion of a single dose of spermine was studied (Wéry and Dandritfosse, 1993; Kaouass et al., 1996; Wéry et al., 1996a). The effect seems to evolve in two phases: some intestinal variables change during the initial few hours of the experiment whereas other intestinal variables are clearly modified about 30 h after spermine administration.

4.1. Initial hours of the experiment

As early as 4 h after spermine ingestion, the weight of intestine per cm of length decreased in parallel with a decrease in DNA weight per cm of length. This resulted from a loss of villus epithelium cells; optical microscopy showed that villus height also decreased drastically. These modifications occurred simultaneously to a decrease in lactase and maltase SAs, especially in the ileum. The ileum was more affected than the jejunum by spermine administration. This observation could be due to the well-known difference in the enterocytes ultrastructure and function of these two parts of the small intestine (Baintner and Veress, 1967).

The oral administration of spermine to suckling rats had no effect on DNA synthesis and had only a slight effect on protein synthesis 3 h after the beginning of treatment. Thus, the growth arrest of intestinal cells can be excluded as the cause of the cell loss observed 4 h after spermine administration. Furthermore, at 5 h post-treatment, the incorporation of $^{14}$C-leucine into proteins isolated from the jejunum increased, indicating that protein synthesis was enhanced at that time.

Spermine is well known to induce apoptosis of different cell-type. Poulin et al. (1995) have shown
that spermine accumulation in cells can induce death. Recently, Stefanelli et al. (1998) demonstrated that spermine can directly activate some caspase and, in this way, induce apoptosis. Furthermore, it is well known that apoptosis occurs in the small intestine in order to regulate cell numbers (Hall et al., 1994). Thus, this phenomenon could explain the observed cell loss (see later). This fact was proven by Peulen et al. (1997, 1998a) by using terminal deoxynucleotidyl transferase-mediated deoxyuracil triphosphate-fluorescein nick end labelling (TUNEL), transmission electronic microscopy, DNA laddering and caspase-3-like activity measurement. These authors have also obtained data indicating that this phenomenon is probably not induced by a cytokine secretion but rather by spermine per se after its accumulation in the enterocytes (Peulen and Dandrifosse, 2000).

The effect of spermine ingestion on the SAs of intestinal disaccharidases was also investigated in cells coming from different parts of the crypt–villus axis (Wéry and Dandrifosse, 1993; Wéry et al., 1996a). Sucrase SA was undetectable in all the categories of isolated enterocytes. Two hours after spermine ingestion, lactase SA increased in the epithelial cells at the top of the villi and decreased in the epithelial cells at the bottom of the crypts. The maltase SA increased in all the epithelial cells, except in those at the bottom of the crypts.

4.2. Thirty hours after spermine ingestion

From 10 to about 48 h after administration of a single dose of spermine to unweaned rats, new enterocyte differentiation appeared in the small intestine. The intestinal weight and DNA weight per cm of length increased, until they were significantly greater than control values. These modifications were observed in parallel with the appearance of the biochemical characteristics of weaning, for example, a change in disaccharidase SA. Lactase SA remained low from the beginning to the end of the experiment. The SA of sucrase became readily measurable 20 h after the spermine treatment and increased until it reached adult values. Spermine induced the sucrase first in the enterocytes at the bottom of the villi, a result confirming that new cell differentiation has to occur before the observation that spermine can induce normal-like intestinal maturation (see later). The SA of maltase increased as for sucrase SA and also reached adult values. The spermine and spermidine levels in the intestine of the spermine-treated rats were generally higher than that of the control animals.

Thus, the early gut atrophy which occurred after the oral administration of spermine was rapidly reversed, taking about 24 h in the jejunum and 48 h in the ileum. The reversal is due to new cell differentiation. However, simple reversal can be obtained in suckling rats after indomethacin (Kabouass et al., unpublished data) or muscimol (El Khefif et al., unpublished data) treatment.

To explain the results obtained above, especially those showing that spermine ingestion induces a desquamation of the villus cells, it may be argued that the effect of exogenous spermine on intestinal variables is due to polyamine toxicity. Such a possibility can be excluded for the following reasons: (1) the spermine dose ingested corresponds to the amount found in the daily solid food eaten by the 25-day-old rats (Pollack et al., 1992; Romain et al., 1992), (2) the amount of spermine administered to the animals is not cytotoxic (Rosenthal and Tabor, 1956), and (3) when spermine is administered to suckling rats at nephrotoxic doses, the animals died within 24 h (Buts et al., personal communication).

Furthermore, the concentration of spermine, which is high in the doses given to the rats (5 to 160 mM; in 50 μl of saline) is quickly diluted in their stomach contents (about 2 ml, i.e., 40 times) and in their chyme (about 2 ml). Moreover, the transfer of spermine from the stomach to the intestine is not the result of a one-time flush. In these conditions, the local concentration of spermine in small intestine is low, all the more so as the concentration of polyamines in the enterocytes is close to 0.5 mM, a concentration of spermine which does not affect the survival of CaCo-2 cells in culture media (Deloyer et al., unpublished results). It could be argued that polyamines are in fact at a concentration below 0.5 mM in the enterocytes due to their associations with anionic components, but this argument is likely to be without substance as polyamines in chyme or in glycocalyx are also combined with anionic molecules. In addition, electron microscopy does not indicate any signs of toxicity in epithelial cells after spermine ingestion. As reported above, apoptosis...
alone seems to be responsible for the desquamation observed.

It may also argued that, in mammalian cells, spermine is converted to acetylspermine and that this is rapidly oxidised by polyamine oxidase (PAO), yielding spermidine and cytotoxic products. The inhibition of PAO by MDL72527, used in order to slow down the metabolism of spermine and thus the synthesis of acetamidopropanal, did not avoid the gut atrophy induced by the ingestion of spermine (Kaouass et al., 1996). Moreover, the intestine of rats treated simultaneously with spermine and MDL72527 appeared more mature than that of rats receiving spermine alone.

These findings prove that spermine itself, without being metabolised to the toxic products mentioned above, is responsible for both cell loss observed at 8 h post-treatment and intestinal maturation observed 3 days later. The physiological state of the cell could be important in this process since the spermine dose used to induce intestinal maturation in suckling rats is without effect on the speed of desquamation of the enterocytes in adult animals (Peulen et al., unpublished data).

5. Direct effect of spermine on the enterocytes

Previous results show that dietary spermine acts in two phases on intestinal properties in suckling rats. To know whether these effects are direct (i.e., concern the enterocytes only) or indirect (i.e., involve hormones, neurotransmitters or local factors), different experiments have been performed. It was found that: (1) in organ culture, spermine had no maturational effect on intestinal explants from suckling rats (Kaouass et al., 1994a), (2) spermine induced maturation in enterocytes isolated from the proximal intestine of suckling rats and cultivated in synthetic media (Wéry et al., unpublished results), and (3) spermine accelerated the differentiation of CaCo-2 cells (Deloyer et al., unpublished results).

Although inconclusive, these observations indicate that spermine could have a direct effect on the enterocytes but do not exclude an indirect effect (see later). At the present time, we do not know whether the polyamine target (specific receptor?) is intracellular or located on the plasma membrane.

6. Dietary spermine and hormonal secretion

Modifications of mature intestinal functions and enzyme expression during weaning are dependent upon genetic, dietary and hormonal factors (Lee and Lebenthal, 1983). The question as to whether or not dietary polyamines act to evoke these changes should be addressed. To answer this question, adrenalectomy was performed (Kaouass et al., 1994b). It reduced the changes in the SAs of disaccharidases induced by dietary spermine. Thus, the intestinal postnatal maturation promoted by spermine could be mediated, at least in part, by the adrenal glands. Nevertheless, adrenalectomy did not completely eliminate the spermine-induced appearance of sucrose and the increase in the SA of maltase (Kaouass et al., 1994b). This may be due to the direct effect of spermine on the enterocytes (see earlier) and/or to factors secreted by tissues other than the adrenal glands. Furthermore, the spermine-induced decrease in the SA of lactase was not affected by adrenalectomy. Thus, adrenal glands are not involved in the spermine-induced decrease of lactase SA observed in vivo studies. Thyroxine and bombesin could be implicated in this phenomenon (see later).

Since parenteral spermine injection had no effect on intestinal postnatal maturation (Kaouass et al., 1994a) and since the adrenal gland is involved in this phenomenon, it may be possible that spermine given orally induces intestinal secretion of one or more substances which, in turn, stimulate the secretion of endogenous local factors and/or hormones. Results have confirmed this possibility (Kaouass et al., 1994b). The ability of spermine to increase corticosterone output was maximal 5 and 6 h after ingestion, a period during which maximal stimulation of adrenocorticotropic hormone (ACTH) output was recorded. This observation suggests that there must be a link between the digestive tract and the pituitary–adrenal axis. This link is not spermine: parenteral spermine administration is ineffective in inducing intestinal maturation and corticosterone secretion (Kaouass et al., 1994a). It could be a gastrointestinal (GI) hormone, a cytokine and/or the gut nervous system (see earlier).

The effects of gastrin, cholecystokinin (CCK-8), vasoactive intestinal peptide (VIP), neurotensin, somatostatin, secretin and glucagon, all intraperitone-
ally injected, were tested (Kaouass et al., 1997a). None of these polypeptides affected brush-border enzyme SA. Thus, these GI hormones do not seem to be involved in spermine-induced intestinal maturation.

There is increasing evidence for a link between the neuroendocrine and immune systems (Rothwell, 1991). Interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α), polypeptides predominantly produced by monocytes and macrophages, have been reported to stimulate the pituitary–adrenal axis by stimulating the corticotropin releasing-factor (CRF)-containing cells in the hypothalamus. In order to assess the possible role of IL-1β, IL-6 and TNF-α as a link between the ingestion of spermine and the activation of the pituitary–adrenal axis, different experiments were undertaken.

The ingestion of spermine produced increases in plasma concentrations of IL-1β, IL-6 and TNF-α (Kaouass et al., 1997b). The peak responses of IL-1β and IL-6, appearing within 6 h after spermine administration, corresponded to the peaks in plasma corticosterone and ACTH concentrations. This ACTH peak could be a result of IL-1β action (Uehara et al., 1987). The increases in plasma concentrations of IL-1β, IL-6 and TNF-α were all observed at the same time; as it has been shown that IL-1β and IL-6 can induce TNF-α release (Wong and Clark, 1988), it cannot be excluded that the action of spermine on TNF-α release was mediated by IL-1β and/or IL-6.

Thus, it may be suggested that spermine ingestion by unweaned rats stimulates the secretion of IL-1β, IL-6 and/or TNF-α, from the gut-associated-lymphoid-tissue (GALT) system. These substances then appear in the general blood circulation and could increase corticosterone secretion, thus triggering the intestinal maturation process. This possibility is highly probable because data (Kaouass et al., 1997b) show that i.p. administration of IL-1β or IL-6 precociously changes the disaccharidase SA and growth of the small intestine, and that cyclosporin A inhibited the increase of the disaccharidase SA induced by spermine ingestion (Peulen and Dandrifosse, 2000). It was confirmed by results showing that intraperitoneal (i.p.) injections of IL-1β and IL-6 increased the concentration of corticosterone in plasma. Nevertheless, a direct action of these cytokines on the enterocytes is not excluded: receptors for IL-6 are present on the human intestinal epithelial cells (Shirota et al., 1990).

From another point of view, there are several reports indicating that IL-1β, IL-6 and TNF-α administration increases ACTH and corticosterone plasma concentrations in adult animals (Rivier et al., 1989; Besedovsky et al., 1991; O’Grady et al., 1993). Such observations were also made in suckling rats, although IL-1β induced a more marked effect than did IL-6 and TNF-α. Assuming that this effect is mediated in the same way as in adult animals, i.e., through increasing CRF release, these results show that the pituitary–adrenal system is already mature enough in suckling rats to respond to the stimulatory effect of immunological factors.

These cytokines also increased the intracellular polyamine concentration (Kaouass et al., 1997b). As IL-1β activates the ODC contained in intestinal epithelial cells in vitro, it is possible that the increase of polyamine content induced by IL-1β and IL-6 is a consequence of a direct action of these cytokines on the intestinal ODC activity. Nevertheless, it cannot be excluded that an indirect action of IL-1β and IL-6 on the intestinal polyamine concentration occurs through an increase in plasma concentration of corticosterone. The latter is known to be an activator of ODC.

The indirect action of spermine, via factors other than corticosterone, on the enterocytes is not excluded: the administration of IL-1β to the rats induced release of insulin and prostaglandins (Uehara et al., 1987), both of which are involved in postnatal intestinal maturation. Cytokines other than IL-1β and IL-6 could also play a role in this effect.

7. Effect of dietary spermine on the nervous system

The nervous system could play a role in the intestinal maturation induced by spermine ingestion. A partial confirmation of this possibility came from an investigation of the effects of bombesin on the small intestine in suckling rats (Kaouass et al., 1997a). Parenteral injections of bombesin (an hormone but also a neurotransmitter) to suckling rats
stimulated growth of the small intestine (i.e., increased intestinal weight and length, and intestinal protein and DNA contents). It also induced appearance of sucrase, an increase in maltase SA and a decrease in lactase SA.

The mechanism by which bombesin affects intestinal development is unknown. Specific receptors to this neuropeptide are present on intestinal enterocytes (Moran et al., 1988). Thus, a direct action of bombesin on enterocyte metabolism cannot be excluded. Bombesin could also modify intestinal functions via endogenous growth factors or hormones, such as polyamines (Grillo, 1985) and corticosterone (Henning, 1981). Indeed, plasma concentrations of corticosterone and intestinal levels of intracellular polyamines were enhanced when bombesin was administered to the animals (Kaouass et al., 1997a). Adrenalectomy in bombesin-treated rats generally decreased the disaccharidase SA.

As the effect of bombesin on corticosterone secretion could be mediated in the same way in adult animals, i.e., through an increase in ACTH release (see earlier), it was questioned whether or not blood and intestinal bombesin concentrations are modified by spermine treatment. It was shown that this polyamine had no apparent action on the concentration of bombesin in plasma (Kaouass et al., 1997a). However, the intestinal content of bombesin was significantly decreased. Since this change in intestinal bombesin coincides with the increase in plasma corticosterone concentration, produced by spermine ingestion, the existence of a relationship between the two phenomena, by the way of the nervous system, was suggested. The more plausible hypothesis is that spermine stimulates the secretion of bombesin from enteric nerves in the synaptic cleft where bombesin could act directly on the enterocytes and/or on a postsynaptic nervous receptor (with, at the end, activation of the pituitary-adrenal axis). Afterwards, this neuropeptide would be rapidly catabolised (“inactivated”).

Based on these findings, the effect of spermine on corticosterone secretion could partly be mediated through an enteric-nerve neurotransmitter-dependent mechanism in which bombesin would be implicated.

The fact that bombesin decreases lactase SA is important since that is the first time that it has been demonstrated that an endogenous factor, other than thyroxine, can affect this parameter in that way. Indeed, injection of hormones as corticosterone (Martin and Henning, 1982), insulin (Buts et al., 1988), prostaglandins (Neu et al., 1983) or insulin-like growth factor-I (IGF-I) (Young et al., 1990), usually enhances the SA of intestinal lactase in suckling animals. These findings suggest that, like thyroxine, bombesin can be involved in the decline of lactase SA during the period of weaning.

Other arguments confirm that that dietary spermine could affect the nervous system of the gut. Putrescine can be metabolised to γ-aminobutyric acid (GABA) and the endogenous phosphorylation of the GABA type A receptor is modulated by spermine (Bureau and Laschet, 1995). In preliminary experiments (El Khefif et al., unpublished results), we showed that spermine modified intestinal motility in suckling and adult rats, and that muscimol, a GABA agonist, produced an intestinal desquamation similar to the type caused by spermine but followed by regeneration without the appearance of adult forms of enterocytes.

Consequently, dietary spermine could act indirectly on the enterocytes, by way of the nervous system, at the first stage of its action in the small intestine.

8. Effect of dietary spermine on the immune system

The immune system could be influenced by spermine ingestion as suggested by Kaouass et al. (1997b) and by Peulen and Dandrifosse (2000) in rats (see earlier: the effects of interleukins on postnatal intestinal maturation). This was confirmed by studies performed in suckling mice. Indeed, Ter Steege et al. (1997) have proven that the percentage of intra-epithelial lymphocytes expressing the T-cell receptor αβ (TCRαβ), clusters of differentiation (CDs)-4, -5 and -54, as well as the levels of expression of these antigens, increased similarly after spermine ingestion to that seen with normal maturation.

The effect of spermine and/or spermidine on human lymphocyte differentiation and proliferation has also been studied. Spermine inhibited DNA synthesis by lymphocytes isolated from the tonsils of children and then stimulated by a mitogen (phytohaemagglutinin L), but only in the presence of bovine serum (El Khefif et al., 1995). In serum-free
medium which supports in vitro proliferation of human T cells stimulated by the same mitogen, spermine induced proliferation of these cells. It also acted on the differentiation of the latter. Indeed, when spermine was added to culture media, a decrease in the expression of the IL-2 receptor (CD25 marker) and of the transferrin receptor (CD71 marker) was observed, whereas the CD4/CD8 ratio was not modified. Under the same experimental conditions, the expression of the CD23 marker of B lymphocytes was not affected.

9. Effect of dietary spermine on postnatal maturation of the pancreas and liver

As reported above, during the third postnatal week, many modifications appear in the digestive system of the rat. They seem to be an adaptation to the progressive transition from a milk regime (high fat, low carbohydrate diet) to a solid regime (high carbohydrate, low fat diet). In the pancreas, modifications occur spontaneously during the first 3 weeks of postnatal life (for references, see Snook, 1971). They are probably under a hormonally-controlled “biological clock” but could also be dependent on food intake. The following modifications occur in rats aged between 15 and 30 days: (1) an increase in pancreatic weight (proportionally higher than the body weight increase) and (2) an increase of acinous cell number compared with Langerhans-islet cells. From a biochemical point of view, normal maturation of the rat pancreas occurs mainly during the fourth week after birth. It is characterised by an increase in α-amylase, trypsin and lipase activities. Morisset and Grondin (1987) have shown that ODC activity and polyamines intervene in the pancreatic growth of neonatal rats, and results are supported by those obtained by Romain et al. (1998).

Romain et al. (1998) treated suckling rats with spermine. Whereas pancreatic DNA and protein contents (mg/g wet weight) were not affected by the treatment, pancreatic weight, and protein and DNA content (per unit body weight) increased significantly. These changes could simply be a function of body weight but in fact rat weight did not decrease during the spermine treatment but rather increased less quickly, at least at the beginning of the experiment, than in control animals. Consequently, almost all the modifications observed in the pancreas after feeding spermine to suckling rats are similar to those observed in normal pancreatic maturation. Thus, it may be proposed that spermine treatment induces maturation of the pancreas in suckling rats as it does for the intestine.

The effect of spermine on intestinal maturation could involve at least the activation of ACTH and corticosterone secretion. As the latter could have an effect on pancreas or liver maturation, in the experiments described below, hepatic properties were analysed after feeding spermine to suckling rats.

Putrescine and spermidine contents were higher in the livers of pups than of adults, while liver spermine content increased slowly with age. After spermine treatment, the putrescine or spermidine content decreased in pup liver until it was close to that measured in adult liver. Administration of spermine for 3 or 5 days to suckling rats induced an almost two-fold increase in liver spermine content which was higher than that found in adult liver. In rats, during the normal maturation, the liver spermidine/spermine ratio decreased progressively with age until it stabilised at about 1:1 in adult animals. The oral administration of spermine to unweaned rats produced a significant decrease in this ratio, which reached a value close to 1:1.

Vaerman et al. (1989) detected only a trace of RPI in the liver of suckling rats but observed that the amounts of RPI started to increase in rats from the age of 20 days, and reached adult values in rats older than 40 days. An increase (almost two-fold) in this variable was recorded after 3 days of treatment with spermine, although the value obtained did not reach the adult one (Wéry et al., 1996b).

The enzymatic activity of ornithine aminotransferase (OAT) is a typical marker of postnatal liver maturation and it markedly increases during weaning (Greengard, 1970). Spermine given orally to suckling rats induced a significant increase in liver OAT activity which equalled that of 21-day-old rats (Wéry et al., 1996b). These data indicate that orally administered spermine initiates liver differentiation but is not sufficient to induce complete liver maturation.

The physiologically important role played by endogenous and exogenous polyamines in the postnatal maturation of the whole gastrointestinal tract (GIT) in the rat was confirmed by the above observations.
10. Molecular mechanism of spermine action on intestinal maturation

In the rat, the most recent findings (see earlier) allow us to present a working hypothesis explaining the effect of spermine on the maturation of the GIT. This particularly depends on results obtained at the level of the intestine. In this organ, two phases characterise the effect of spermine. A desquamation of the intestinal epithelium is followed by a hormonal cascade.

In the early phase, spermine first enters the enterocytes where it induces apoptosis. This latter phenomenon does not seem to be influenced by cytokines, even if the secretion of these substances increases. Secondly, the high permeability of the intestinal epithelium, characteristically observed in suckling rats, allows spermine to enter into the intercellular space where it reacts with different receptors located in the nervous system, in the immune system and on the endothelial cells at the capillary ends. The nervous system target is not yet identified but we know that spermine can be transformed into GABA and can act directly on the GABA receptor. Results also suggest that bombesin neurosecretion is affected. Thirdly, as a consequence, intestinal motility is decreased, as observed in vitro in rat pups and as reported in adult animals. Fourthly, a stagnation of biliary salts in the lumen of the intestine appears, speeding up the desquamation of the cells at the top of the villi. Fifthly, spermine and maybe aldehydes, derived from polyamines by metabolism, act in the immune system or on epithelial cells, where they induce the secretion of various cytokines such as IL-1β, IL-6 and TNF-α. This could provoke, among other things, the production of nitric oxide and some oedema (noted at the top of the villi), a phenomenon subsequently reinforced by an inhibition of ATPase activities by spermine. This oedema provokes the start of inflammation (as has been observed) but this phenomenon is antagonised by spermine. Although not yet conclusively demonstrated, the oedema and inflammation seem to be side effects of spermine action since neither anti-IL-1β and anti-TNFα antibodies nor cyclosporin A prevent the desquamation of the intestinal epithelium (Peulen and Dandrifosse, 2000).

Following the above sequence of events, a hormonal cascade occurs. The more plausible hypothesis, resulting from established observations, is that spermine stimulates the secretion of bombesin from enteric nerves in synaptic clefts; the bombesin then acts directly on the enterocytes and/or on postsynaptic nerve receptors with, eventually, activation of the pituitary–adrenal axis. Besides bombesin, IL-6, IL-1β and maybe other not yet identified cytokines could also indirectly affect this axis. As the nervous system and cytokine secretions are stimulated, the c-fos gene is activated, and ACTH and corticosterone are secreted. Corticosterone increases insulin and thyroxine secretion, and, since insulin affects the permeability of the intestinal cells to spermine and since the effect of polyamine on the DNA expression is well known, it may be inferred that another pattern of enterocyte differentiation occurs, which is similar to the adult stage. The increase in the spermine intracellular concentration results also from a direct entrance of spermine into the enterocytes and from an indirect effect of corticosterone on the ODC activity of the stem cells.

The molecular mechanism at the basis of the maturation of the pancreas and of the liver after spermine ingestion originates mainly from the hormonal cascade reported above.

When normal maturation occurs, the same phenomena take place (Peulen et al., 1998b). They are less marked and difficult to show, except for the increase in spermine, the secretion of several hormones (corticosterone, TNF-α, insulin), the variation in polyamine concentration in the enterocytes, the modification of gene expression, apoptosis and oedema (Peulen et al., 1997, 1998a; unpublished results). The irreversibility of the normal process is unclear but could be due either to the biological clock (often cited as the basis of the intestinal maturation process) or to the changing hormonal status observed after weaning.

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