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Review

Relation of β-casomorphin to apnea in sudden infant death syndrome

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Abstract

Sudden infant death syndrome (SIDS) is the most common cause of death in infants and its pathogenesis is complex and multifactorial. The aim of this review is to summarize recent novel findings regarding the possible association of β-casomorphin (β-CM) to apnea in SIDS, which has not been widely appreciated by pediatricians and scientists. β-CM is an exogenous bioactive peptide derived from casein, a major protein in milk and milk products, which has opioid activity. Mechanistically, circulation of this peptide into the infant's immature central nervous system might inhibit the respiratory center in the brainstem leading to apnea and death. This paper will review the possible relationship between β-CM and SIDS in the context of passage of β-CM through the gastrointestinal tract and the blood–brain barrier (BBB), permeability of the BBB to peptides in infants, and characterization of the casomorphin system in the brain.

Keywords: β-Casomorphin; Sudden infant death syndrome; Blood–brain barrier; Peptides; Exogenous peptides; Peptidase; Opioid; Opioid receptor; Respiratory center; Apnea

1. Introduction

Sudden infant death syndrome (SIDS) is defined as the sudden death of an apparently healthy infant less than 1 year of age, which remains unexplained after a thorough case investigation, including performance of a complete autopsy, examination of the death scene, and review of the clinical history. SIDS is the most common cause of death in infants between 1 month and 1 year of age [13]. The etiology of this tragic event includes maternal factors such as narcotic addiction, young age, poor nutrition, and smoking during pregnancy. Other factors include multiple births, pre-maturity, and heredity. A subset of children who develop SIDS display a functional abnormality of the pontomedullary respiratory center and autonomic nervous system, which may result in severe apnea or sudden death [91]. Airway obstruction due to reflux or respiratory infection may contribute to a child's susceptibility.

One factor that is common to all children who develop SIDS is that milk is their main food source. Infants fed either formula preparations or human milk have a similar risk of developing SIDS. Milk is comprised of a heterogeneous protein mixture that can be separated, characterized, and classified into two major components, casein and whey proteins. Caseins are the main milk proteins in cow's milk, contributing 78–80% of the total protein. In human milk, they constitute only 20–30% of the total protein [34]. Casein can be fractionated into α-, β-, and κ-caseins. Opioid peptide β-CMs, representing fragments of a larger protein called β-casein, have been isolated and identified in fresh cow and human milk, as well as in dried infant formula [14,35]. After ingesting any form of milk or milk products, smaller peptides like β-CMs are formed in the stomach of infants by the gastric enzymatic breakdown of casein [22]. Once β-CMs are formed, they are fairly resistant to proteolysis because of their proline-rich sequence, and thus may reach significant levels in the stomach. Following absorption from the gastrointestinal (GI) tract, β-CMs can easily cross the blood–brain barrier (BBB) because of the infant's immature central nervous system. In infants with abnormal respiratory control and vagal nerve development, the opioid peptides derived from milk might induce depression of the brain-stem respiratory centers, leading to apnea and death. Thus, opioid peptides derived from milk might be an additional factor contributing to the incidence of apnea and SIDS.

Abbreviations: SIDS, sudden infant death syndrome; GI, gastrointestinal; BBB, blood–brain barrier; β-CM, β-casomorphin; CSF, cerebral spinal fluid

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2. Passage of casomorphin through the GI tract

In the study of Svedberg et al. [85], considerable amounts of β-CM (1–7), β-CM (1–4), and β-CM (1–6) but not β-CM (1–5) were found in in vitro digests of bovine milk, and in small intestine contents after bovine milk ingestion in adult humans. However, it remains to be clarified which type of CM is present in more caudally located sections of the GI tract. Demonstration of immunoreactive bovine β-CM material in the GI tract raises the question of whether these agents should be regarded as "food hormones" with a local action in the bowel [65]. However, the possibility that they might be absorbed intact into the blood and subsequently into the central nervous system or other organs to elicit opioid effects also must be considered. Although β-CMs are known to be highly resistant to proteolytic enzymes, inactivation is rapidly accomplished by the enzyme dipeptidyl-peptidase IV [51], which is present in the intestinal mucosa [21], meconium [21], plasma [48,51], and other tissues like placenta, exocrine glands, and kidneys [21]. This enzyme cleaves dipeptide fragments (X-Pro) from the N-terminus of peptides [21,51]. However, studies on immunoreactive β-CM in blood after ingestion of milk failed to detect such opioid peptides in adult human volunteers [89]. In newborn calves, plasma β-CM (1–7) immunoreactivity was detected after the first milk intake following birth [90]. This material was identified as an extended peptide and a probable precursor of β-CM (1–7), which was protected at the N-terminus against enzymatic degradation by dipeptidyl-peptidase IV [90]. Thus, peptide sequences with potential opioid activity seem to be absorbed through the GI wall of newborn mammals [49,90,93], but are less likely to appear in the circulation of the normal adults. It cannot, however, be excluded that exogenous opioid peptides derived from food products could be absorbed more readily in subsets of individuals of the population or in some individuals with pathophysiological conditions involving the GI tract. If bovine or human β-CMs occur in the circulation after intestinal absorption, they must localize in the central nervous system in order to elicit either respiratory or endocrine effects. That the level of casein-derived β-CM7 is elevated significantly in urine [20,47,78,79] and blood [56,57,79] of patients with schizophrenia, autism, and postpartum psychosis clearly indicates that this bioactive peptide crosses the GI mucosa and enters blood to a significant degree in certain individuals. The mechanism by which this occurs is not fully understood. It may be related to genetically pre-determined peptidase defects (or deficiency) and/or increased intestinal permeability [20]. Thus, the level of circulating β-CM7 may be elevated due to the deficiency of metabolizing enzymes and/or intestinal disorders. It is important to note that the enzyme or peptidase activity of the GI tract is almost non-existent in pre-weanlings, allowing the absorption of peptides and proteins from milk [1,10,37,66,76,84,90].

3. Role of the kidney in uptake of β-casomorphin (β-CM)

The kidney plays an important role in determining concentrations and half-lives of circulatory proteins and polypeptides that are smaller in size than albumin. The discriminatory filtering capacity of the glomeruli and the ability of tubular cells to metabolize filtered proteins and peptides largely contribute to this important physiological function of the kidney. Many studies, both in vivo and in vitro, have demonstrated that there are two mechanisms by which proximal tubular cells limit the excretion of the peptide nitrogen. Large molecular weight proteins are reabsorbed via luminal endocytosis followed by intra-lysosomal digestion to peptides and amino acids [58,60]. Small linear oligopeptides, on the other hand, are degraded in the lumen of the brush border where peptidases produce amino acids and smaller peptides containing two to four amino acids, which then are absorbed by carrier-mediated processes [30]. Peptidases found in the renal brush border membrane represent a very potent set of enzymes capable of hydrolyzing a polypeptide to a mixture of small peptides and amino acids [18]. Among these peptidases, dipeptidyl-peptidase IV is unique in a number of ways. It is the only enzyme that belongs to the class of "serine peptidases" in the renal brush border membrane, it generates dipeptides in a sequential manner (not amino acids like other amino-peptidases) from large polypeptides, and its substrate specificity is very selective. The major function of dipeptidyl-peptidase IV is to cleave β-CM, which is not cleaved by any other peptidases in the kidney. β-CM is hydrolyzed in the extravesicular medium by the brush border dipeptidyl-peptidase IV to a mixture of Tyr-Pro, Phe-Pro-Gly, Phe-Pro and Gly, and the resulting peptides are then transported into vesicles by the peptide transport system [30].

4. BBB permeability

Substances may cross the BBB in one of three basic ways, denoted as non-specific, transmembrane diffusion, or saturation. The BBB exists because of tight junctions between the endothelial cells of the capillary bed of the brain. This effectively bonds the individual cell membranes together, almost eliminating the plasma ultrafiltrate produced by Starling's forces in most other capillary beds. A few areas of the brain, such as the choroid plexus and the circumventricular organs, have fenestrated capillaries, but these areas are delimited by ependymal cells, possessing tight junctions that impede diffusion to the rest of the central nervous system [92]. Pinocytotic activity across the brain endothelial cell also is relatively reduced. Ultrafiltration and pinocytosis, representative of the non-specific mode of the BBB transfer, are relatively non-discriminatory among substances with regard to molecular characteristics such as size, charge and shape. Albumin is thought to cross the

BBB primarily by non-specific mechanisms and is found in the cerebral spinal fluid (CSF) at 0.5% of its plasma concentration. This demonstrates the restrictive characteristics of the BBB. Broadwell and Sofroniew reported that albumin enters the brain via extracellular pathways [17].

The ubiquity of tight junctions in the capillary bed of the brain produces, in essence, a continuous cell membrane. Therefore, the BBB, like the cell membrane, often behaves like a semi-permeable membrane [62]. The second general way in which substances may cross the BBB is transmembrane diffusion with selective discrimination among substances. Properties such as lipid solubility, charge, size, and shape are of major importance in determining the passage across the BBB. Membrane composition also is a contributing factor. Lipid solubility and molecular weight of a substance are usually the most important factors in determining the degree of crossing [55]. Many centrally active drugs gain access to the central nervous system by this mechanism.

The third general way in which substances cross the BBB is by carrier-mediated transport, which also is a property of cell membranes. This process utilizes protein complexes capable of binding and moving substances across the membrane. These systems are limited in the rate at which material may be transported, and so are saturable. The transport may be energy dependent or independent, may or may not be able to transport substances against a gradient, may be dependent on cofactors for maximal activity, and may have allosteric sites for their regulation. The system tends to be highly specific for a group of closely related compounds. Amino acids, thyroid hormones, hexoses, and organic acids could be transported in this way.

5. The BBB and its permeability to peptides

Some studies and reviews have concluded that the penetration of peptides across the BBB is either absent or negligible [24,26,71,72]. Early studies often gave varying answers due to the lack of appropriate methodologies [68]. In the 1980s, most authorities interpreted the evidence as being against the passage of peptides. Reviews now and then include statements such as: “Thus it is unlikely that distribution of peptides in brain will occur after systemic administration” [71]. Subsequent studies have confirmed that the BBB excludes peptides: “In conclusion, the diffusion of most peptides across the brain vascular endothelium seems to be severely restricted” [59]. “Circulating peptides cannot penetrate the BBB owing to the paucity of pinocytosis and to the presence of high-resistance tight junctions joining virtually all brain capillary endothelia” [18,70]. The above ideas suggest, however, that many early studies did not fully account for the complexity of the BBB. For example, some studies have argued that a lack of correlation between levels of peptide in the lumbar CSF and blood is evidence against the passage of peptides across the BBB. Classic work done with non-peptide substances shows that it is more appropriate to use CSF from the lateral ventricles or posterior fossa in such assessments [19,31]. In general, peptide levels in the CSF obtained from more rostral locations correlates best with serum levels of peptide [2]. In addition, the short half-life of peptides in the circulation coupled with apparently low BBB permeability make it difficult to use many of the more sophisticated techniques to quantify passage.

An alternative explanation of the literature suggests that most peptides do cross the BBB to a low, but probably biologically relevant degree [2]. The extent of passage for most of the peptides examined was based on their physicochemical properties, especially lipophilicity [3]. This suggests that transmembrane diffusion is the major mechanism underlying permeability of the BBB to peptides. The best-studied example of this is the delta sleep-inducing peptide which, when injected into the blood, crosses the BBB to enter the CSF of the dog [11] and brain tissue of the rat [6,7,9,10,42,43]. Entry into the CSF appears to be mainly non-saturable [9], which suggests that the more lipophilic analogs penetrate to a greater degree [8] and have a more profound effect on the electroencephalogram [64].

Although the permeability of most peptides seems to fit the transmembrane diffusion model, a few peptides do not. In a survey of 18 peptides, a few were found to have a lower degree of permeability than predicted by their lipophilicities [3]. These peptides had molecular weights under 10,000 Da, N-terminal tyrosines, tended to have high lipophilicities, and had a lower number of charged sites. This group included β-CM (1–5) and β-CM (1–4) [29,45,46]. Further investigation showed that a saturable, highly specific brain-to-blood transport system exists for these peptides [5]. Thus, the BBB plays an important role in determining the relationship between the levels of substances in the central nervous system and in the peripheral circulation. Peptides, usually distinguished from larger proteins (e.g. albumin) and glycoproteins, are produced by both peripheral organs and the central nervous system, and influence events in both neural and non-neural tissues [39,50]. In addition, reviews have emphasized that peripherally administered peptides can influence central nervous system events such as electroencephalographic activity and behavior [41,44]. Other studies have shown that centrally administered peptides may influence events in peripheral organs [28,53,75,88]. Several mechanisms not involving direct BBB transfer of peptides have been proposed, and some studies have elucidated which peptides on one side of the BBB may influence events on the other [44,63]. Nevertheless, direct movement of peptides remains the simplest mechanism for the transfer of information across the barrier. If peptides cross the BBB, then such passage may play a role in brain–body communications. Alternatively, if peptides normally do not cross, then conditions in which the BBB is altered or disrupted could allow abnormal passage to occur, thereby producing some of the manifestations of disease.

6. Characterization of the casomorphin system in brain

Most studies in this system have been performed in vivo with anesthetized animals receiving intra-ventricular injections [67] of radioactively iodinated inhibitors with or without candidate inhibitors. Animals then were decapitated and transport determined based on the residual counts in the brain. Transport of β-CMs and related peptides out of the central nervous system also has been demonstrated in rats and mice. In both species, β-CM (1–5) Tyr-Pro-Phe-Pro-Gly or methionine enkephalin inhibited the disappearance of radioiodinated β-CM from the brain, but β-CM (1–4) Pro-Phe-Pro-Gly or the amino acid tyrosine did not. An excess of unlabeled β-CM (1–5) increased the half-time disappearance of radioiodinated β-CM (1–5) from the brain of mice from 14 to 88 min [12]. The maximal transport rate V_{max} for β-CM (1–5) was estimated by two different methods to be 0.266 nmol/g (brain) min and 0.297 nmol/g min with K_ms of 15.2 and 15.1 nmol/g. Preliminary studies indicated that the V_{max} of methionine enkephalin was 0.630 nmol/g min with a K_m of 24.9 nmol/g. The levels of methionine enkephalin were about 85 times higher than the levels of β-CM (1–5) in 4-month-old Fisher 344 rats [4].

To induce central effects, blood-borne β-CM (1–5) must either pass through the BBB to reach essential elements of the brain, or alter the BBB transport of agents essential for brain function [27]. The uptake of β-CM (1–5) was measured in 18 brain regions and in the anterior pituitary shortly after intra-carotid administration. The accumulation of radioactivity significantly exceeded that of a non-diffusible reference. Furthermore, the accumulation of radioactivity was independent of the concentration of the injected peptide.

The CSF concentration of β-CM7 was significantly elevated in patients with postpartum psychosis compared with healthy lactating women [57], indicating that β-CM7 penetrates the BBB. It has been reported that low amounts of β-CM5 are taken up by BBB protected areas of the brain after intra-carotid injection, suggesting that it can cross the BBB [27]. β-CM might easily cross the BBB of patients with infantile autism because of the developmental immaturity of the central nervous system. In view of the metabolic stability of this peptide, its accumulation may become considerable. It is generally accepted that enzymatic degradation of the natural β-CM in CSF occurs less rapidly than in plasma [15]. Sun et al. reported that intravenous administration of human β-CM7 at different doses to rats induced moderate to strong Fos-like immunoreactivity in discrete brain regions which was blocked by pre-treatment with naloxone [87]. Further studies from this laboratory have shown that intra-peritoneal administration of β-CM7 caused behavioral changes in rats [86]. These results clearly indicate that β-CM7 can cross the BBB. The mechanism by which β-CM penetrates the BBB is not clear. Peptides and larger proteins (insulin, transferring, cationized albumin, angiotensinogen, etc.) can cross the BBB by the receptor-mediated peptide transport mechanism [69]. Leucine and methionine enkephalins can enter the brain in significant amounts via the specific enkephalin transport system in the BBB [42].

7. Casomorphins and SIDS

Although naturally occurring β-CM (1–5) and β-CM (1–7) exhibit potent opioid activity upon intra-cerebroventricular administration in adult rats and newborn rabbits, only the amidated C-terminus structure, morphiceptin, which is smaller and less polar, elicited ventilatory effects in the adult rat. Administration of high doses of bovine β-CM (1–5) failed to elicit respiratory effects in rabbits. Most studies have found that the newborn rodent BBB does not have increased permeability to proteins. Nevertheless, Peruzzo et al. reported that there is a progressive development of the barrier between the median eminence and the acuate nucleus in the rat during the first postnatal weeks, suggesting that the brain barrier is not fully developed in the newborn rat [74]. The area postrema, a circumventricular organ that lacks a BBB, contains respiratory neurons [80,83] and has projections to the rat rostral ventral respiratory center [40]. It is well known that human infants have an immature BBB. Thus, entrance of β-CM to the central nervous system would be expected to occur more readily in infants before the BBB is fully developed.

Bovine and human β-CMs bind specifically to the μ-opioid receptor [14,15,86,87]. C-terminal amidation of the natural bovine β-CM (1–4) and β-CM (1–5) increases binding to the μ-receptors in the brain, but does not appear to change the low binding affinity to δ- and κ-receptors [15]. Moreover, introduction of D-alanine into position 2 in the β-CM sequence markedly increases the δ-binding affinity without influencing the μ-opioid activity [15]. The results of respiratory studies agree with the view that bovine β-CM (1–5) and β-CM (1–7), as well as morphiceptin, are primarily μ-agonists. They behave similarly to morphine and the effects are readily reversed or prevented by administration of naloxone, a preferential μ-antagonist. However, the reported potency of β-CMs as respiratory depressants varied, in contrast to previous studies [14,15]. It was found that bovine β-CM (1–5) was approximately 10 times more potent, while β-CM (1–7) and β-CM (1–4) were equipotent to morphine.

Most studies of opiate drugs and respiration imply action on chemo-sensitive structures in the brain stem and the carotid bodies. However, in addition to this, a documented effect on intrinsic neuronal pools rostral to the brain stem is certainly suggested [82]. A long-lasting depression of ventilation was seen after intra-cerebroventricular but not systemic administration of CM (1–5) and CM (1–7), which would suggest a central site of action. However, the amidated form of CM (1–4) induced respiratory effects after systemic administration. These effects were characterized by naloxone reversibility, relatively short-lasting depression of the tidal volume and an increase in respiratory frequency.

A similar type of breathing pattern was described for morphine, and is related, in certain species, to enhancement of the Hering-Breuer reflex [61]. The possible site of action for such an interaction remains to be elucidated. An alternative explanation for the ventilatory pattern seen after peripheral morphiceptin administration may be the activation of opioid receptors in the carotid body. Further studies involving local intra-cerebral administration of these peptides, as well as electrophysiological recordings of carotid sinus nerve activity after systemic administration of β-CM (1–4) amide, are needed to finally resolve this question.

Several lines of evidence indicate that endogenous opioids may have a tonic influence on breathing, especially during early neonatal life [82]. Furthermore, endogenous opioids also may have a more significant influence on central respiratory regulation during acute illness or hypoxemia as β-endorphin concentrations in plasma increase during such conditions [38]. In fact, increased plasma, as well as cerebrospinal fluid, β-endorphin concentrations have been found in infants where apnic breathing patterns were found [38,54,81]. It has been reported that β-CM immunoreactivity was found in the brain stem of the human infant [73]. Thus, β-CM has been suggested as a possible cause of SIDS [77]. This is apparently unrelated to its peripheral effect, if any. In addition, intra-peritoneal administration of μ-receptor-selective enkephalin analogues induces an irregular breathing pattern and apnea in newborn pre-term rabbit pups [36]. Thus, enkephalins and endorphins might play a role in both normal and hypoxic breathing in the newborn [32,33]. The opioid antagonist, naloxone, may decrease the duration of the primary apnea in experimental neonatal asphyxia [23], and also lessen the decrease in ventilation which may be elicited by hypoxia in newborn infants [25]. There is no convincing evidence that increased opioid activity is related to SIDS. However, the similarities between the hyperendorphin syndrome [16] and the near miss SIDS has led to speculation that excessive endogenous opioid activity might be one of many causes of SIDS [52]. An extension of this premise would be that excessive exogenous opiod activity by β-CM may induce or add to endogenous opioid tone to impair breathing in the neonate. Actually, exogenous peptides inhibit peptidase/enzyme, which increases the activity of endogenous opioid. Thus, bovine or human β-CMs may be of pathological importance in some infants with SIDS who may absorb excessive amounts of such bioactive peptides. In order to establish such a hypothesis, however, endogenous as well as exogenous opioid peptides must be measured in plasma and CSF of neonates with respiratory disturbances and compared to concentrations in healthy individuals.

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