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MINI-REVIEW

Delta sleep-inducing peptide (DSIP): a still unresolved riddle

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Abstract

Delta sleep-inducing peptide (DSIP) was isolated from rabbit cerebral venous blood by Schoenenberger-Monnier group from Basel in 1977 and initially regarded as a candidate sleep-promoting factor. However, the link between DSIP and sleep has never been further characterized, in part because of the lack of isolation of the DSIP gene, protein and possible related receptor. Thus the hypothesis regarding DSIP as a sleep factor is extremely poorly documented and still weak. Although DSIP itself presented a focus of study for a number of researchers, its natural occurrence and biological activity still remains obscure. DSIP structure is different from any other known representative of the various peptide families. In this mini-review we hypothesize the existence of a DSIP-like peptide(s) that is responsible (at least partly) for DSIP-like immunoreactivity and DSIP biological activity. This assumption is based on: (i) a highly specific distribution of DSIP-like immunoreactivity in the neurosecretory hypothalamic nuclei of various vertebrate species that are not particularly relevant for sleep regulation, as revealed by the histochemical studies of the Geneva group (Charnay *et al.*); (ii) a large spectrum of DSIP biological activity revealed by biochemical and physiological studies *in vitro*; (iii) significant slow-wave sleep (SWS) promoting activity of certain artificial DSIP structural analogues (but not DSIP itself!) in rabbits and rats revealed by our early studies; and (iv) significant SWS-promoting activity of a naturally occurring dermorphin-decapeptide that is structurally similar to DSIP (in five of the nine positions) and the sleep-suppressing effect of its optical isomer, as revealed in rabbits. Potential future studies are outlined, including natural synthesis and release of this DSIP-like peptide and its role in neuroendocrine regulation.

Keywords: delta sleep-inducing peptide (DSIP), neuropeptides, sleep regulation.
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Delta sleep-inducing peptide (DSIP), a nonapeptide with a unique amino-acid sequence (Table 1), was isolated from the cerebral venous blood of rabbits subjected to low-frequency ('hypnogenic') electrical stimulation of the intralaminar thalamic nuclei (the so-called 'trophotropic zone'; Hess W. R. (1944, see: Monnier and Schoenenberger, 1983, for reference) by Schoenenberger-Monnier group from Basel in 1977 (see Monnier and Schoenenberger, 1983; Schoenenberger, 1984, for reference). In the following three decades, extensive studies carried out in a number of laboratories (the total DSIP bibliography could be estimated at not less than 1500 references; Myers 1994) have demonstrated that DSIP, or some structurally closely related peptide(s), is present in both free and bound forms in some cerebral structures, primarily the hypothalamus and limbic system, as well as pituitary and different peripheral organs, tissues and body fluids, where it co-localizes with several peptide and non-peptide mediators (Graf and Kastin 1984, 1986; Schoenenberger 1984; Kovalzon 1986, 1994; Inoué and Schneider-Helmert 1988; Yehuda *et al*. 1988; Inoué 1989; Bjartell 1990; Inoué and Kruger 1990; Prudchenko and Mikhaleva 1994; Lysenko and Mendzheritsky 1995; Strekalova 1998; Pollard and Pomfrett 2001).

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Abbreviations used: ACTH, adrenocorticotropic hormone; ANP, atrionatriuretic peptide; CGRP, calcitonin gene-related peptide; CLIP, corticotrophin-like intermediate lobe peptide; DSIP, delta sleep-inducing peptide; DSIP-LI, DSIP-like immunoreactivity; ICV, introcerebroventricular; MCH, melanin-concentrating hormone; MSH, α-melanocyte-stimulating hormone; NPY, neuropeptide-tyrosin; PS, paradoxical sleep; SWS, slow-wave sleep; TSH, thyreotropic hormone.

Table 1 Structure of delta sleep-inducing peptide (DSIP) and dermorphins (homologueous residues are stressed)

Peptides	Amino-acid sequence
	N 1 2 3 4 5 6 7 8 9 10 C
DSIP	Trp---Ala-Gly-Gly-Asp-Ala-Ser---Gly-Glu
Dermorphin	Tyr-D-Ala-Phe-Gly-Tyr-Pro-SerNH_2
Analogue-1	Tyr---Ala-Phe-Gly-Tyr-Pro-Ser---Gly-Glu-Ala
Analogue-2	Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser---Gly-Glu-Ala
Analogue-3	D-Tyr---Ala-Phe-Gly-Tyr-Pro-Ser---Gly-Glu-Ala

Analogue-1, dermorphin decapeptide analogue liberated after enzymatic digesting of prodermorphin expressed in mammalian cells *in vitro* (Seethaler *et al*. 1993); analogue-2, dermorphin-decapeptide isolated from the skin of a tree frog *Phyllomedusa sauvagei* (Montecuchi *et al*. 1981); analogue-3, artificial dermorphin-decapeptide analogue.

Unlike the initial works that used antibodies of low sensitivity, the histochemical studies of the Geneva team published in the late 1980s and early 1990s used a batch of polyclonal as well as monoclonal antibodies that are highly sensitive and selective mostly to the $DSIP_{5\text{-}9}$ epitope (Charnay *et al*. 1992). These studies revealed the precise localization of DSIP-like immunoreactivity (DSIP-LI) in hypothalamic and adjacent neurosecretory nuclei (which are not particularly relevant for sleep regulation) of a variety of mammals as well as cold-blooded vertebrates. Considerable interspecies differences have been found. Thus, DSIP-LI is present in the hypothalamus of cartilaginous fish (dogfish, *Scyliorhinus canicula*; Vallarino *et al*. 1992), rat, guinea pig, rabbit, cat and human. In the mammalian hypothalamus DSIP-LI co-localizes with the peptide hormones luliberin (gonadoliberin) and oxitocin/neurophysin-1 (Bjartell 1990; Kovalzon 1994); however, DSIP-LI is absent in hypothalamic median eminence of mouse, hamster and gerbil (Charnay *et al*. 1992). In the pituitary of vertebrates DSIP-LI co-localizes with such peptides as corticotrophin-like intermediate lobe peptide (CLIP), adrenocorticotropic hormone (ACTH), melanocyte-stimulating hormone (MSH), thyreotropic hormone (TSH) and melanin-concentrating hormone (MCH), and it co-localizes with adrenaline and noradrenaline in the adrenal medulla (Table 2). The human pituitary does not appear to be the site of synthesis of plasma DSIP-LI (Friedman *et al*. 1994a). DSIP-LI is abundant in secretory cells of the gut in the rat, pig and human, where it co-localizes with the following peptides: in the upper part of the gut (antro-duodenic mucosa), gastrin/cholecystokinin; middle part (small intestine), secretin; lower part (large intestine), peptide tyrosine—tyrosine (PYY)/glycentin. In the insular isle of the pancreas in rabbit, pig and human, DSIP-LI is co-localized with the peptide glucagon. DSIP-LI is detected in considerable quantities in malignant tumour cells of the adrenals and intestine, and in the latter it is co-localized with serotonin (Bjartell 1990; Kovalzon 1994). In general, despite some discrepancies, these data could be hardly regarded as insufficiently convincing. It should be mentioned however that the immunohistochemistry does not prove that the peptide is naturally spontaneously released. Electronic microscopy demonstrating the presence of the peptide in vesicles located in nerve terminals would be highly suggestive. The release of the peptide might also be measured by microdialysis and HPLC, which would be highly challenging.

Table 2 DSIP-like immunoreactivity (DSIP-LI) representation and co-localization in pituitary and adrenals of various vertebrates

Subject		Pituitary			Adrenal medulla
		anterior	intermediate	posterior	
Man	1	++	+	+	++
	2	CLIP			adrenaline
Pig	1	+	++	+	++
	2	ACTH/MSH			noradrenaline
Cat	1	+	++	–	
	2	ACTH/MSH	ACTH/MSH		
Rabbit	1	+	++	–	+
	2	ACTH/MSH	ACTH/MSH		
Rat	1	+	–	++	++
	2	TSH			noradrenaline
Mouse	1	+	–	++	–
	2	TSH			
Frog	1	++	++		
	2	ACTH	ACTH		
Dogfish	1	++	++		
	2	MCH	MCH		

1: ++, dense; +, sparse; –, absent. 2: – Co-localization; ACTH, adrenocorticotropic hormone; CLIP, corticotrophin-like intermediate lobe peptide ($ACTH_{18-39}$); MCH, melanin-concentrating hormone; MSH, α-melanocite-stimulating hormone ($ACTH_{1-13}$); TSH, thyreotropic hormone (Vallarino *et al*. 1992; Yon *et al*. 1992; Kovalzon 1994).

Biochemical studies both *in vivo* and *in vitro* revealed a low level of DSIP molecule stability. After administration from the outside DSIP molecule degrades rapidly under the influence of a specific aminopeptidase-like enzyme, primarily by splitting off the N-terminal amino-acid residue of Trp and the subsequent Ala, and so the half-life of the DSIP

molecule *in vivo* is not more than several minutes (Graf *et al.* 1984, 1986; Schoenenberger 1984; Inoué *et al.* 1988; Yehuda *et al.* 1988; Bjartell 1990; Prudchenko *et al.* 1994; Strekalova 1998). Theoretical calculations predicted and physico-chemical studies confirmed various folded conformations of the DSIP molecule in water solutions, as well as the lipophilic surrounding (David 1994; Gray *et al*. 1994; Kovalzon 1994; Polverini *et al*. 1998), which break down following the splitting off of the N-terminal Trp that apparently leads to peptide inactivation. In the organism, endogenous DSIP possibly exists mainly either by complexing with a protective carrier protein preventing it from degradation, or as a component of at least three high-molecular-weight precursors the structure of which remains unknown. Endogenous DSIP could possibly be present in its native form as well as in phosphorylated and glycosylated forms (Bjartell 1990), which suggest the existence of a DSIP-like peptide family.

Despite its rather large size, the amphiphilic DSIP molecule can partly penetrate the brain–blood barrier as well as cellular membranes; both passive diffusion and a specific transportation mechanism localized in the area of the floor of the 4th ventricle have been proposed (Graf *et al.* 1984, 1986; Schoenenberger 1984; Inoué *et al.* 1988; Yehuda *et al.* 1988; Bjartell 1990; Gray *et al*. 1994; Augustijns *et al*. 1995; Polverini *et al*. 1998; Strekalova 1998). Permeability changes in relation to circadian rhythms and is possibly dependent on the functional state of an organism (Kovalzon 1986, 1994; Yehuda *et al.* 1988).

Thus it appears that endogenous DSIP [or DSIP-like peptide(s)] is a typical regulatory peptide and should therefore play an important role in endocrine regulation. Indeed, although data on the direct effects of DSIP upon noradrenergic, serotonergic and GABA-ergic transmission in the brain are not consistent (Lysenko *et al.* 1995; Strekalova 1998), it was shown that DSIP:

(i) decreases the basal corticotropin level and blocks its release evoked by corticoliberin injection (Graf *et al.* 1984, 1986; Schoenenberger 1984; Kovalzon 1986, 1994; Yehuda *et al.* 1988; Bjartell 1990; Inoué *et al.* 1990; Chiodera *et al*. 1994; Prudchenko *et al.* 1994; Lysenko *et al.* 1995; Strekalova 1998; Pollard and Pomfrett 2001);

(ii) stimulates the secretion of luteinizing hormone;

(iii) stimulates the release of somatoliberin and somatotropin and inhibits somatostatin secretion (Kovalzon 1994).

So the endocrine functions of DSIP, rather than its immediate effects upon neurone transmission, are likely to determine, at least partly, the role it plays in a broad spectrum of both physiological and pathological processes, some of them not directly related to others:

(i) responses to stress, where DSIP acts as a previously unknown stress-limiting factor (Sudakov *et al*. 1983; Graf *et al.* 1984, 1986; Schoenenberger 1984; Kovalzon 1986, 1994; Inoué *et al.* 1988, 1990; Yehuda *et al.* 1988; Inoué 1989; Bjartell 1990; Prudchenko *et al.* 1994; Lysenko *et al.* 1995; Strekalova 1998; Gershtein and Dovedova 1999; Pollard and Pomfrett 2001);

(ii) immune responses, where it can play the role of immunomodulator, cell differentiation factor and anti-tumour agent (Prudchenko *et al*. 1993b; Fabien *et al*. 1994; Heritier *et al*. 1994; Popovich *et al*. 2003);

(iii) DSIP has also been shown to have hypothermic properties (DSIP was demonstrated to have an enchanting effect on serotonin-, apomorphine- and opioid-induced hypothermia; Tsunashima *et al*. 1994) and a complex effect on thermoregulation (Yehuda *et al*. 1980; Graf *et al.* 1984, 1986; Schoenenberger 1984; Yehuda and Mostofsky 1984; Yehuda *et al.* 1988; Bjartell 1990; Strekalova 1998; Pollard and Pomfrett 2001). A close correlation between the diurnal rhythm of DSIP-LI and that of body temperature was detected. It was concluded that endogenous elevation of DSIP may be associated with suppression of both SWS and paradoxical sleep (PS), and that the circadian rhythm of DSIP is coupled directly or indirectly to that of body temperature (Friedman *et al*. 1994b; Schulz *et al*. 1994; Seifritz *et al*. 1995; Steiger and Holsboer 1997);

(iv) cardiotropic and vasomotor activity (DSIP can normalize blood pressure and myocardial contraction; Schoenenberger 1984; Yehuda *et al.* 1988; Inoué 1989; Prudchenko *et al.* 1994; Strekalova 1998);

(v) pain, where DSIP can produce an analgesic effect by augmenting met-encephalin binding with opiate receptors (Schoenenberger 1984; Yehuda *et al.* 1988; Inoué *et al.* 1990; Prudchenko *et al.* 1994; Lysenko *et al.* 1995; Strekalova 1998; Pollard and Pomfrett 2001);

(vi) regulation of diurnal and circadian rhythmicity (Friedman *et al*. 1994b; Schulz *et al*. 1994; Seifritz *et al*. 1995; Vgontzas *et al*. 1995);

(vii) anti-opioid and anti-alcohol activity (DSIP can suppress the development of alcohol and opiate dependency this being the basis for DSIP clinical application; Yukhananov *et al*. 1991, 1992; Soyka and Rothenhaeusler 1997; Backmund *et al*. 1998; Hruz *et al*. 2001; Khvatova *et al*. 2003);

(viii) anticonvulsive effects (Prudchenko *et al*. 1993a; Stanojlovic *et al*. 2002, 2004);

(ix) antioxidant effects (Prudchenko *et al.* 1994).

Thus, summing up all these separate and contradictory data, one can suggest that endogenous DSIP [or DSIP-like peptide(s)] is nothing but a previously unknown humoral regulator of the body carrying out various functions, the role of a hypothalamic ‘trophotropic hormone’ and stress-limiting factor being primary among them. This ‘optimistic’ point of view is contradicted, however, by the ‘skeptical’ one based, in its turn, on the following: (i) the lack of detection of a DSIP precursor protein(s) structure; (ii) the lack of detection of a DSIP precursor protein gene; (iii) the lack of detection of a DSIP specific receptor; (iv) the lack of detection of a DSIP

receptor gene; (v) the lack of detection of a DSIP amino-acid sequence in DSIP-like immunoreactive material (Bjartell 1990; Sillard *et al*. 1993; Prudchenko *et al.* 1994; Strekalova 1998). One possible source for criticism was the method used for DSIP amino acid sequence determination, which may result in possible errors. The isolation and chemical determination were performed only once and were not checked by modern methods. So it was proposed that the identification of an isolated nonapeptide was erroneous, and instead an endogenous DSIP-*like* peptide(s) existed in mammals (Kovalzon 1994, 1999; Strekalova 1998). Thus, there is some doubt that the synthesized DSIP studied in all of the following biological investigations is really identical to the natural peptide from sleep-inducing rabbit dialisate.

The skepticism concerning the natural occurrence of DSIP was increased by the contradictory results in studies of DSIP biological activity (Yon *et al*. 1992), at least *in vivo* (Späth-Schwalbe *et al*. 1995), and especially on sleep. A number of studies failed to confirm SWS- or PS-promoting properties of DSIP, as well as an increase in the EEG spectrum delta power, after either introcerebroventricular (ICV) or systemic administration of DSIP; only minor effects were found in others, and even an impairment of sleep has been reported (Schneider-Helmert and Shoenenberger 1983; Obál *et al*. 1985; Kovalzon 1986, 1994, 1999; Obál *et al*. 1986; Borbely and Tobler 1989; Inoué 1989; Elkafi *et al*. 1994; Strekalova 1998). The link between DSIP and sleep has never been further characterized, in part because of the above-mentioned lack of isolation of the DSIP gene and protein and a possibly related receptor. Thus, the hypothesis regarding DSIP as a sleep factor is extremely poorly documented and is still weak. At the same time, our earlier studies using a number of DSIP structural analogues (synthesized by V. N. Kalikhevich and S. I. Churkina, University Chemical Institute, St. Petersburg, Russia, and I. I. Mikhaleva and I. A. Prudchenko, Institute of Bio-organic Chemistry, Russian Academy of Sciences, Moscow) have demonstrated an evident SWS-promoting activity of several of them. These active analogues had a substitution of the N-terminal Trp residue by its D-isomer or the isomer of D-Tyr; additionally, the substitution of the Ala residue at the second position by its D-isomer or D-Val isomer had a similar effect. This means that the increase in DSIP molecule resistance against aminopeptidase degradation did lead to the clear appearance of its functional (sleep-promoting) activity *in vivo*. These data were obtained in joint totally blind studies of the Russian, Hungarian and Japanese researchers of the late 1980s (Obál *et al*. 1986; Kimura-Takeuchi *et al*. 1990; Kovalzon 1994, 2001). The following experiments revealed that conformation of a molecule plays an important role too: studying a pair of the analogues, [*N*-MeAla^2]DSIP and [β-Ala^2]DSIP, both possessing higher aminopeptidase resistance but different flexibility of the peptide bone (rigid in the former and flexible in the latter one), we discovered strikingly different effects. Thus, the peptide [*N*-Me-Ala^2]DSIP increased the SWS percentage within a 12-h recording period following ICV administration of 25 nmol, whereas [β-Ala^2]DSIP decreased it. The impression was that the above-mentioned pair of DSIP structural analogues plays the role of the 'agonist–antagonist' ligands to some as yet unknown receptor.

To resolve the paradoxical problem of the natural occurrence and biological activity of DSIP, we proposed the existence of some as yet unknown peptide of which the entire amino-acid sequence is similar although not identical to that of DSIP. This molecule could be responsible (at least partly) for the binding with both anti-DSIP antiserum and DSIP putative receptor(s). This idea was supported by the discovery of a dermorphin-like decapeptide (Seethaler *et al*. 1993; analogue-1, Table 1). In these *in vitro* experiments, the authors used a recombinant vaccine virus expressed in mammalian cells, the cDNA coding for preprodermorphin precursor that processed further to prodermorphin. After its enzymatic digesting, a new peptide was liberated. The authors noted some similarity of this new decapeptide to bradykinin and luteinizing hormone, but were not concerned with its similarity to DSIP. Using the peptide bank EROP-Moscow (Zamyatnin 1991a,b) we discovered the close similarity of this peptide to DSIP (in five of the nine positions, see Table 1), which was especially evident in the C-terminal site of the molecule. The dermorphin-decapeptide molecule has three aromatic hydrophobic amino acid residues separated from each other by linear non-polar radicals. These structural features suggest a high potency of the peptide for binding with membrane receptors and activating intracellular processes; therefore, high biological activity of the analogue 1 could be anticipated.

Dermorphins are a well-known family of the peptides isolated from amphibian skin; a unique characteristic peculiar to their structure is the presence of D-Ala residue at the second position, which makes the dermorphin molecule highly resistant to aminopeptidase activity (Table 1). Dermorphins are the products of enzymatic digestion of the precursor protein prodermorphin followed by secondary processing that results in the transformation of the second residue of L-Ala into its optical isomer (Kovalzon 1999; Kovalzon *et al*. 2002; Usenko *et al*. 2002). Although such processing is not known for mammalian tissue, at least the analogue-1 (Table 1) built of L-amino acid residues could be present there and bind to anti-DSIP antibodies, especially taking into account that most of them recognize the C-terminal part of the DSIP molecule. 'Classical' dermorphin-heptapeptide (Table 1) is a potent agonist of the opiate μ-receptors and thus possesses very high analgesic properties. However, the way in which dermorphins effect sleep was unknown.

We carried out a study of three dermorphin-decapeptide analogues in our rabbit test system (Kovalzon *et al*. 2002).

All three analogues (one of them, analogue-2, has a natural origin as it was also initially isolated from the skin of a tree frog as well as the 'classical' dermorphin-heptapeptide) had an identical structure but differed in their hirality (Table 1). The peptides were synthesized by V. N. Kalikhevich and Z. A. Ardemassova (University Chemical Research Institute, St. Petersburg, Russia). Strikingly different results have been found: whilst dermorphin analogue-1 (as well as DSIP itself) failed to show any influence upon sleep following the administration of 30 nmol ICV, dermorphin analogue-2 significantly increased the SWS percentage during the post-injection hours 7–11, and dermorphin analogue-3 decreased it. Again, as it was in the case of DSIP structural analogues (see above), the pair of aminopeptidase-resistant dermorphin analogues play the role of 'agonist-antagonist' ligands of an unknown receptor depending upon the conformation of the analogue. This putative receptor could be hardly regarded as a μ-opioid one, because analgetic activity of dermorphins is almost immediate to their administration and lasted not more than half an hour. Thus, it is possible that analogue-1, if endogenously present in mammalian organism, is responsible for binding with anti-DSIP antibodies, whereas analogues-2 and -3 being injected from the outside, are responsible for the demonstration of ligand–receptor interaction and biological (sleep-promoting) activity (Kovalzon 1999; Kovalzon *et al*. 2002). A long delay between ICV administration of the peptides and their effects upon sleep suggests an indirect influence that is probably mediated through some hormonal axis.

The idea that the biological activity of DSIP is 'hidden' and cannot be demonstrated in a number of studies as a result of its fast degradation received additional support in our recent study with a combined administration of DSIP, neuropeptide-tyrosin (NPY), atrio-natriuretic peptide (ANP) and calcitonin gene-related peptide (CGRP). These combinations have been predicted theoretically as possessing anxiolytic properties that should be more expressive than the same of each of the peptides administrated separately (Koroleva and Ashmarin 2002). As anti-phobic effects (Strekalova 1995) usually associate with stress-protective and hypnogenic effects, we carried out the combined ICV administration of DSIP + NPY, DSIP + NPY + ANP and DSIP + NPY + CGRP. We found that the 'ineffective' DSIP could potentiate the sleep-promoting activity of NPY, ANP and CGRP within the first 6-h period after administration (Kovalzon and Fesenko 2003; Kovalzon *et al*. 2003).

Concerning the presented data, we would like to conclude that there is still no strong evidence of the natural occurrence of DSIP. However, the results permit us to suppose the existence of some still unknown DSIP-like peptide(s) structurally related to DSIP and, probably, to the above-mentioned dermorphin-decapeptide analogue-1 (Table 1). From the described findings we suggest that the potential way to clarify the question of the natural occurrence of DSIP might be the investigation of analogue-1 including its role in neuroendocrine regulation. Additionally, it cannot be excluded that physiological and immunocytochemical studies would lead to the discovery of more DSIP-like peptide(s). We believe that the problem of DSIP physiological activity is mainly a result of the lack of data on the direct interaction of DSIP with any kind of receptor. Hence, we suggest that the simultaneous registration of the physiological effects of DSIP/dermorphin and the detection of its binding sites in the same experiments might be more successful in the search for the mechanism of action of DSIP. Finally, as follows from the proposed conclusions, the definitive solution to both the problems of DSIP natural occurrence and its physiological activity needs new and extensive investigations. We feel that despite the objectively skeptical view on the questions discussed, there is enough background and motivation to be encouraged to perform the studies leading to the definitive answer.

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