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Delta-Sleep-Inducing Peptide (DSIP): A Review

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GRAF, M. V. AND A. J. KASTIN. *Delta-sleep-inducing peptide (DSIP): A review.* NEUROSCI BIOBEHAV REV 8(1) 83–93, 1984.—Since the turn of the century, it has been postulated that humoral factors induce sleep. Many compounds were proposed as sleep-factors, but only two of the sleep-peptides have been purified to homogeneity and characterized, so far. One of them, DSIP, was shown to be a nonapeptide of MW 849 and to induce mainly delta-sleep in rabbits, rats, mice, and humans, whereas in cats, the effect on REM sleep was more pronounced. A U-shaped activity curve was determined for the dose as well as for the time of infusion. DSIP-like material was found by RIA and immunohistochemistry in brain and by RIA in peripheral organs of the rat as well as in plasma of several mammals. In addition to sleep, the peptide also has been observed to affect electrophysiological activity, neurotransmitter levels in the brain, circadian and locomotor patterns, hormonal levels, psychological performance, and the activity of neuropharmacological drugs including their withdrawal.

Sleep-factors DSIP Sleep Physiological functions Stress EEG Neuropharmacological agents

DELTA-sleep-inducing peptide (DSIP) was isolated, characterized, and synthesized more than 6 years ago. In the meantime, about 100 publications have dealt with this peptide but most of the following major questions are still open: what are its main functions? Is it really a sleep factor? Does it occur naturally? How does it exert its activities? This review evaluates the currently available knowledge about DSIP.

DSIP was first proposed as a sleep-peptide [130] and this remains the prevalent impression of its function. Since normal people spend about one third of their lifetime sleeping (about 25 years), it is rather surprising that the phenomenon of sleep and its purpose, which is still in question [45], have been long overlooked as a topic of scientific interest. A decisive step forward was made between 1928 and 1938 when H. Berger described the electroencephalogram (EEG) of man [6,7]. After this, the EEG became the main tool used for the characterization of sleep and, subsequently, electrostimulation of the brain became the predominant way to influence it. Sleep induction by intracranial electrostimulation represented the "dry way" compared to the "wet way" by infusion of humoral factors into the body. A broad variety of such factors have been shown to induce sleep or sleep-like behavior, including small organic molecules like tryptophan as well as macromolecular proteins [13–15, 42, 67, 74, 86, 122, 148, 150, 152, 155].

The isolation and characterization in the brain of peptides regulating the release of hormones at very low concentrations, and thus influencing basic body functions [64], has encouraged the search for similar factors responsible for sleep-control. Only one decade ago, the prevalent view was that sleep regulation was merely a question of the cooperation of the different neurotransmitter systems, especially serotonin (5HT [50]). In recent years, however, the idea has become widely accepted that peptides may play an important role in the organization of sleep, although these functions are not yet clarified [68,96].

SLEEP-INDUCING PEPTIDES

The first to answer the question "What induces sleep?" with the suggestion of a humoral factor were Legendre and Pieron [80,82]. They reported the possible existence of "hypnotoxin" in the blood, cerebrospinal fluid (CSF), and brain of dogs after sleep deprivation for 6–15 days. Intracerebroventricular (ICV) injections of serum and CSF from sleep-deprived dogs induced behavioral sleep (determined by visual observation) in normal dogs. The proposed sleep-factor was water soluble, thermolabile (65°C), sensitive to oxidation, and not dialyzable [81,82]. Part of these findings was confirmed in 1939 by Schnedorf and Ivy [121], and other groups later isolated sleep-factors from brain and body fluids of sleep-deprived animals.

For instance, Drucker-Colin and his group perfused cat brains with a push-pull cannula after 24 hr sleep-deprivation and infused the perfusate into cats that were made alert by food-deprivation [21]. The perfusion liquid increased the time of slow-wave-sleep (SWS) and reduced the latency to the first SWS episode. No effect was observed for paradoxical sleep (PS) [19]. In their later work, they reported the isolation and partial characterization of two proteins apparently involved in rapid eye movement (REM) sleep [141], a finding supported by the increase in REM-sleep after infusion of antibodies generated against these proteins [20].

Pappenheimer reported in 1967 that an infusion of CSF from sleep-deprived goats reduced the locomotor activity of

rats [109]. This group later extracted, isolated, and partially characterized a substance called factor "S" [77,108]. ICV infusions of this factor also induced prolonged SWS in rats and rabbits. It seems to be a peptide with a molecular weight of about 500 [28]. Krueger, from the same group, extracted a factor with similar properties from human urine (urinary sleep-promoting factor: SPU) [76]. The final characterization revealed that SPU was a small glycopeptide containing glutamic acid, alanine, diaminopimelic acid, and muramic acid in molar ratios of 2:2:1:1 [79]. It, therefore, resembles a bacterial peptidoglycan, muramyldipeptide. Similar analogs, generally used as immunological adjuvants [5], had pyrogenic properties strongly correlated with the SWS-inducing activities [75]. Recent attempts showed that these two activities could be dissociated, at least partially [78]. No effect of factor "S" nor SPU have been reported on paradoxical sleep or circadian rhythms.

In 1977, Pavel and his group published that arginine-vasotocin (AVT), a nonapeptide first isolated from the pineal, induced SWS in cats after ICV infusions [113]. The doses used to elicit these sleep effects were remarkably low (10^{-6} pg). The onset of action occurred within 5 min and lasted for about 30 min. At the same time, however, PS was suppressed for 5 hr [113]. These authors suggested that the effects of vasotocin on sleep involve serotonin-containing neurons [111]. ICV injections of AVT antiserum enhanced the amount of REM sleep by increasing the REM periods and reducing REM latency [112]. Subcutaneous (SC) injection in human beings enhanced PS [48]. Some of these results have been confirmed by others [90,147].

Another sleep-factor thought to be a peptide was isolated from brainstems of sleep-deprived rats by Nagasaki *et al.* [103]. The sleep-promoting substance (SPS) seems to contain two or more active fragments which increased both SWS and PS in recipient rats and mice [48,149]. Furthermore, SPS-infusions significantly reduced locomotor activity during darkness and increased SWS in rats. Such effects lasted for two days [44,47]. SPS also affected the circadian organization of activity and sleep [48]. For more details about endogenous sleep-promoting factors, the reader is referred to an excellent short review published by Inoue, Uchizono, and Nagasaki [48].

Most recently, Jouvet and coworkers screened several peptides including angiotensin II, arginine vasotocin, substance P, neurotensin, beta-endorphin, enkephalins, and cholecystokinin-octapeptide for a sleep-inducing effect. Although only vasoactive intestinal peptide (VIP) showed such an action [117], this represents the decisive step from the idea of a merely monoaminergic regulation of sleep to a more comprehensive view of sleep as a biological function.

ISOLATION AND CHARACTERIZATION OF DSIP

The basis for the isolation of a humorally transmitted sleep-factor was the demonstration of its direct transport from one animal to another through connected blood vessels. In fact, parabiotic rats showed high synchronization in their sleep pattern, especially in REM sleep [88]. As early as 1961, Kornmueller *et al.* reported that cats showed sleep EEG after their blood vessels were connected to their counterparts in other cats, which were electrically stimulated to sleep [69]. Monnier *et al.* found similar results with rabbits in 1963 [100]. These experiments constituted a major breakthrough because they showed clearly that normal sleep could be transmitted humorally. They also involved the use of sleeping animals as the source of a sleep factor. This is in contrast to most other workers searching for natural sleep-factors with preferentially sleep-deprived animals. This may have been a reason why, some years later, another group could not find any sleep-inducing activity in reconstituted plasma of sleep-deprived rabbits [116]. Sleep deprivation probably constitutes a strong stress situation which was largely avoided by Kornmueller and Monnier.

Further evidence for a sleep-inducing factor in blood was provided by the demonstration that hemodialysates of sleep-stimulated rabbits increased delta-sleep in recipient rabbits [98–100]. The increase in delta waves of sleeping rabbits was further used to isolate a "sleep-inducing factor delta." It was known from dialysis to be a small substance, and most of the common electrolytes were excluded [100]. Monnier and Schoenenberger combined the efforts of their groups isolating the "factor delta" and reported in 1972 that it seemed to be a peptide of 700 MW with tryptophan or serine as the N-terminal amino acid and an effective dose (ICV) of 5–8 ng/kg [97, 101, 130, 131]. Shortly afterwards, the peptide was shown to contain 9 amino acids, including tryptophan, with an apparent molecular weight of about 860 [132]. Furthermore, the term "organizing mediator on neurotransmitters" was introduced to better explain the effects of the peptide [135]. In 1977, final determination of the sequence of natural DSIP and its synthesis were reported, and the effects of the synthetic and the natural peptides were compared [94, 102, 133, 136]. This "delta-sleep-inducing peptide" was shown to have the following sequence: Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu, with a molecular weight of 848.98.

The purified peptide produced an enhancement of delta waves in rabbits of about 43% after the first hour [133]. The same sequence, synthesized in solution, elicited a 39% (limbic) to 54% (neocortex) increase of the delta activity and a significant increase in spindle activity [136]. It is noteworthy that short DSIP analogs like Trp-Ala-Gly-Gly, Asp-Ala-Ser-Gly-Glu and Trp-Ser-Glu also had some activity whereas long analogs like DSIP 1–8, 2–8, 2–9, Arg^3-Tyr^5-DSIP, and Arg^3-Gly^5-DSIP did not [136]. Slightly different short analogs did not show a sleep-inducing effect in a different laboratory [83] and the peptide with a β-bond at the asparagine in position 5 was not able to produce any significant sleep effects [134,137]. Besides the usual synthetic methods [49, 59, 92, 106], artificial genes for this peptide were also prepared [70] and incorporated into suitable vectors that were cloned to yield high amounts of DSIP determined by RIA [18]. Conformational analysis of synthetic DSIP by spectral methods revealed that the peptide probably occurs in aqueous solution as a folded structure with extended polar groups, the central position of β-COOH inside [92]. It remains to be clarified if the structural conformation of DSIP in solution is related to its biological activity.

Other studies revealed an inverted U or bell-shaped dose-response curve [137,146]. Such curves have been shown for other peptides, too [22, 56, 61, 66]. It is now widely accepted that many peptides do not exhibit a saturation curve for the dose-response relationship. The optimal effect of DSIP in inducing delta-waves was found at about 7 nmoles/kg (ICV) and about 30 nmoles/kg (intravenous [IV] injection) [137], but differences may be observed even with different infusion sites in the brain [146]. The fact that at each step of the isolation the active fraction could be determined only in a more dilute solution of the substance represents one of the intriguing features of the isolation of DSIP.

This probably is a manifestation of the inverted U-shaped dose-response curve.

More recently, it was shown that another type of bell-shaped curve may exist for the time of IV infusion similar to that seen with dose [125]. An infusion for 1 min did not produce any sleep effect, whereas a duration of 2.5 and 7.5 min enhanced sleep more than an infusion for 20 min. However, this result was obtained in humans, and the temporal as the qualitative organization of sleep in rabbit and human is apparently quite different.

OCCURRENCE AND METABOLISM

Though a direct proof of the existence of natural DSIP is still lacking, several observations in addition to its isolation suggest the occurrence of such a peptide or a structurally related molecule in vivo. These include the demonstration that considerable amounts of DSIP-like material were found in rat brain by radioimmunoassay (RIA) [59]. Gel chromatography of the acid-extracted material showed a peak of immunoreactivity at the position where synthetic DSIP eluted [59]. Immunoreactive DSIP-like material was also reported in the fetal rat brain [57]. However, more advanced steps are needed to firmly establish the existence of DSIP or a closely related structure.

Recent attempts in our laboratory to determine DSIP-like material in peripheral organs of the rat showed immunoreactive material throughout the body in the range of several hundred pg per mg wet tissue [36]. These unusually high levels were obtained after extraction with water. Although an artifact due to enzymatic degradation of the tracer can probably be excluded, other possibilities have to be taken into account. One of these might be an interfering substance of unknown origin. Another explanation could be that the structure of DSIP is bound to larger molecules as proposed for DSIP occurring in plasma of mammalian species [55]. Addition of DSIP to plasma increased the large molecular form of immunoreactive material and treatment with acid reduced it. Gel chromatography of the extracts of the rat organs also showed most immunoreactive material to be larger than DSIP. Degradation of the DSIP-like immunoreactive material (DSIP-LI) with trypsin did not destroy the immunoreactivity but produced small fragment(s) still reacting in the RIA. Part of this material eluted at the position of DSIP when chromatographed on Sephadex G-25. Dilution of the organ extracts to less than 1 mg wet weight tissue per ml increased the percentage of DSIP-LI which could be removed by charcoal adsorption before the RIA. This could indicate a dissociative process. A coating of the charcoal with protein preventing the adsorption of DSIP at these low concentrations is not very likely, and it would not explain the increasing effect of charcoal preadsorption on the DSIP levels at higher tissue concentrations. However, other possible artifacts may exist.

Small amounts of DSIP-LI that eluted at the same position as the synthetic peptide have been found in all mammalian plasma tested [55]. Further indications for a binding process were obtained when DSIP was injected into dogs and the apparently free DSIP in the plasma remained unchanged while the DSIP-LI with an apparent large molecular weight was markedly enhanced [3]. Under the same conditions in CSF, a larger peak of small peptide was found to elute at the same position as DSIP. It may be that only free DSIP passes the blood-brain barrier into the CSF where less "binding protein" seems to exist, but where small DSIP-LI has also been measured in the basal state [3]. The existence of DSIP-LI in plasma and CSF has been confirmed recently by Ekman *et al.* [24] who developed an RIA for DSIP using slightly different techniques.

We also found DSIP-LI in human breast milk [35]. This may be relevant to the finding that neonatal mammals can absorb DSIP in intact form through the gastro-intestinal tract [4]. Since the passage of DSIP and its analogs through the blood-brain barrier has been demonstrated [53, 58, 60], one could speculate that DSIP in breast milk can influence the sleep-wake cycle of neonates by absorption through the gastro-intestinal tract and penetration of the blood-brain barrier. In fact, the peptide has been shown to influence circadian rhythms [39,138] and DSIP levels in plasma also seem to change with the time of day [55]. Although seasonal variations in the amounts of DSIP-LI material in brain have been seen, no indication for an important role in the regulation of hibernation was found in one study [73].

Further indications for the occurrence of DSIP-LI in rat brain were provided by immunocytochemistry in two independent studies [26,144]. As was observed by RIA, DSIP-LI was found throughout the brain. Specificity was shown by pre-adsorption of the antibody with the synthetic peptide. The distribution of DSIP shown in these studies suggests that the peptide may be a component of several systems ranging from locomotion to memory, so that even an involvement in psychoses or Alzheimer's disease might be considered [12,27].

In an attempt to further localize DSIP in the body, tritium-labeled DSIP was injected into the tail vein of rats (unpublished data, H. P. Lorez, M. Graf, G. Gillessen, and G. A. Schoenenberger). The distribution of the label was comparable to the results obtained by RIA, but labeled tryptophan injected as a control was found to distribute in a similar pattern. However, the pineal gland seemed to be an exception. First, it showed a considerably higher accumulation of DSIP than the other organs and, though tryptophan was concentrated in that organ too, the concentration of DSIP was higher than the amino acid alone during the first 15 min. Second, several organs were checked by thin layer chromatography for the presence of intact (labeled) peptide, but only the pineal showed up to 30% unchanged DSIP even after 15 min. It is not known if the presence of the highest amount of the label in the pancreas was due to a special affinity for this organ or to a high metabolism of the peptide. Microautoradiography after IV or ICV injection of ^{3}H-labeled DSIP showed a somewhat even distribution of the radioactivity over most brain regions.

Several studies have shown that small amounts of peripherally injected DSIP or its analogs can pass the blood-brain barrier in rats or dogs [2, 3, 53, 58, 60]. But, as found there [3], degradation of the peptide, in addition to other processes like binding, is likely to occur. It is known the DSIP and its analogs can be degraded by brain extracts of rat [46] and mouse [87]. Tryptophan was cleaved at a rate at least twice as fast as any other amino acid or dipeptide and after 15 min at least 50% of this N-terminal amino acid was split from the peptide. However, this fast degradation rate was obtained with brain homogenates or extracts, differing greatly from in vivo conditions, and was not as fast as occurred with degradation of enkephalin [46].

To summarize, DSIP seems to be an ubiquitously occurring peptide in mammals, appearing mostly in large molecular forms. No definite proof has been obtained, as yet, whether the DSIP-like material is in fact DSIP or a closely

related structure. The pineal gland may have a special affinity for this peptide. No specific site of synthesis is known, but the peptide seems to be excreted through the kidney into urine where DSIP-LI has been determined by RIA (unpublished observation and [24]). Since no storage mechanism has yet been found and since rather high endogenous levels of DSIP-LI seem to exist, the question is raised how it is possible for relatively small amounts of injected peptide to induce the effects that are discussed in the following sections.

ELECTROPHYSIOLOGICAL EFFECTS OF DSIP

Sleep-EEG

DSIP was given its name because of its delta-wave (-sleep) increasing effect. The increase of these waves (4 Hertz) in the EEG of rabbits was used for the isolation of the peptide [133]. Until now, this has been the most prominent effect of DSIP. It has been confirmed by other groups in rabbits [83,145], rats ([51, 145, 151], S. Inoue: personal communication), and mice [104], and observed to persist for several hours. According to results published by Karmanova *et al.* [52] and Medvedjev *et al.* [89], DSIP injected ICV at higher doses (20 nmol/kg and more) produced narcosis-like effects in different animals but smaller doses did not yield enhanced sleep effects. This partly supports the findings of several other groups [72, 76, 91, 147] that failed to detect any sleep enhancing effect of DSIP at concentrations of 30–160 nmol/kg IP or 6–24 nmol/kg ICV. It is not known if the applied concentrations were too high or other factors were involved. More detailed studies by a group in Shanghai showed marked increases in delta and sigma activities of rabbits after ICV (5 μg/rabbit) or IV (50 μg/kg) application of DSIP [49,84]. The same group reported similar potencies of a Phe^5 analog as well as of some other analogs [83,85].

Surprisingly, DSIP produced a more pronounced increase of REM sleep than of delta-sleep in cats [114]. According to Borbely and Tobler [10], the finding of an effect of DSIP on more than one sleep form would not be consistent with a natural sleep-factor, since they postulated that the effects of such a compound should be the same in different species with respect to the time course and the sleep states [10]. At 300 nmol/kg, however, no effects of DSIP were detected [114]. In man, enhanced NREM sleep as well as REM sleep was found. Although the effects were consistent and statistically significant, the difference from the normal sleep of these normal subjects was not dramatic [30,124]. Further effects of DSIP on human beings are discussed later.

Although several groups have performed sleep-inducing experiments with DSIP, the results remain controversial. It can be assumed, therefore, that other groups failed to find any sleep-enhancing activity of the sleep-inducing peptide. This failure may be influenced by several factors. As shown earlier in this review, the effects of DSIP require an optimal dose [137,146] as well as an optimal time of infusion [125]. Further, the content of endogenous DSIP seems to follow a circadian rhythm [55] and probably also a seasonal variation [73], and the time of day of the injection can have a considerable influence [31]. An additional variable is that DSIP may not induce its effects directly; a modulating trigger mechanism or other indirect effect should be considered because of the substantial time lag generally occurring between injection and onset of action [8, 31, 51, 95, 127]. As yet, there is little support for such a mechanism of this peptide.

Peripheral injections of DSIP in rats increased the 4 Hertz frequency but at the same time other peptides like α-MSH, endorphins, and enkephalins showed a similar effect [93]. The action of DSIP seemed to show more specific enhancement of the delta-waves than the other peptides (except MSH). The rather weak overall effect of DSIP may be explained by the high dose applied (80 μg/kg, i.e., about 95 nmol/kg), but the effect of DSIP seemed more marked (and specific at 4 Hertz) than that of the other peptides with the exception of β-endorphin [93].

It is possible that the peripheral injections produced their central (EEG) effects indirectly through peripheral factors. But, with the use of isolated rat heads, a shift of the EEG power spectrum toward delta frequencies (2–4 Hz) was observed that was not seen with the β-aspartyl coupled compound [137]. This suggests that there is little or no peripheral influence on the effects of DSIP on the EEG.

Other Electrophysiological Actions

In addition to effects on the nictitating membrane of the cat [9], influences of DSIP on the neurons of the snail, Helix lucorum, were reported. At a concentration of 5×10^{-9} M or more, DSIP reduced the spontaneous firing rate of certain neurons by 30–40% and produced a hyperpolarization of the resting membrane potential [118]. Some analogs showed similar effects but one analog, amidated at Glu^9, showed opposite activity. Recently, Normanton and Gent provided evidence for a direct action of DSIP on single neurons in the nucleus reticularis gigantocellularis of the brain stem in rats and rabbits [105]. In the rat, 56% and, in the rabbit, 71% of the cells showed excitatory action in response to DSIP when applied by microiontophoresis. About 6% of the cells in the rat were inhibited by DSIP. The responses were short lasting, dose-dependent, and without desensitization to repeated application. No correlation was found with similar effects of arginine-vasotocin, thus showing a different mechanism of action for both peptides.

BEHAVIORAL EFFECTS

Early experiments with the synthetic peptide injected IV showed in addition to the increase of the delta waves a concomitant, slight, but significant decrease of locomotor activity [95]. In another report, the number of rightings of rats were counted for the first 3 hours of their natural active phase in the evening. IV injection of 30 nmol DSIP/kg reduced the number of rightings significantly [137]. Later, it was shown that DSIP influenced the circadian activity rhythm of rats. Injection of 30 nmoles DSIP/kg into animals kept in a normal day-night schedule produced a relative decrease of their activity during the dark (active phase) and a corresponding increase during the light (sleep phase). The changes became more pronounced and more consistent after 3–4 days of consecutive injections [34]. Under continuous light resulting in an equal distribution of activity over 24 hr, the effect was greatly enhanced and the onset of action reduced [32]. Under the light/dark regimen, a much smaller dose of DSIP-phosphate, an analog phosphorylated at the serine in position 7, showed a more marked effect with quicker onset than DSIP itself whereas under continuous light, the effects of both peptides were similar. However, the dose of DSIP-P was 300 times smaller than that of DSIP [32,34].

ICV injections of DSIP apparently reduced motor activity of mice from 15–45 minutes after treatment [110]. D-Ala^4-DSIP significantly reduced the swimming activity of

goldfish but DSIP itself failed to do so [107]. In a passive immobility test in which rats were forced to swim, DSIP did not reduce the swimming activity of the rats but D-Ala^3-DSIP had a marked effect [65]. These effects of the D-Ala-analogs of DSIP may be due to a much slower degradation rate than that of DSIP itself [87].

Another action of DSIP that has great potential is its apparent protection against stress. When rabbits were exposed to barking dogs, their EEG showed a distinct disturbance of the sleep pattern for the next 3 hours. Administration of 50 μg/kg DSIP before the stressful confrontation prevented the disturbance in sleep [119]. More recently, Sudakov *et al.* tested the resistance of rats to acute emotional stress induced by electrical stimulation of the ventromedial hypothalamus and of the skin of the immobilized animals. According to the cardiovascular reactions, the rats were categorized into 3 types: resistant, adapted, and predisposed to stress. DSIP, 60 nmol/kg, infused through catheters 15 min before the start of the stress increased the resistance of the rats to emotional stress and decreased the cardiovascular responses [143]. Better coping with stress produced by this peptide in human beings is discussed later.

DRUG INTERACTIONS

DSIP seems to exert effects not only by itself but also appears to interact in various ways with several pharmacological substances. For instance the decrease in temperature of mice injected with alcohol was enhanced after pretreatment with DSIP (unpublished). An interaction between DSIP and alcohol was reported recently by Burov *et al.* [11]. These authors showed an increase of the endogenous level of DSIP in brain determined by RIA after a single injection of alcohol in an inverted U-shaped dose-response pattern. Chronic exposure to ethanol for 12 months, but not 2 months, reduced the amount of DSIP in heavy drinkers significantly [11]. It remains to be confirmed whether this reflects a compensatory mechanism to the rise after single injections as proposed by these authors.

A clear interaction between DSIP and morphine was found several times. A report by Scherschlicht *et al.* in 1979 first described a model of insomnia in cats when the animals were aroused by intraperitoneal (IP) injection of 0.1 mg/kg morphine. Systemic pre-infusion of 30 nmol DSIP/kg completely reversed the arousal induced by morphine, an effect achieved in a comparable manner only by flunitrazepam [120]. Additional evidence of such an interaction was provided by Tissot in 1981. He found enhancement of SWS in rabbits after microinjection of DSIP into different brain regions. These effects were blocked by ICV injections of 160 μg naloxone/kg. It was concluded that DSIP may be a neurotransmitter or modulator of the bulbothalamic system with actions like a morphine agonist [146]. We also found DSIP to reduce the temperature decrease in mice provoked by morphine injections (unpublished). These interactions of DSIP with alcohol and morphine may be relevant to the results obtained with DSIP in the treatment of withdrawal symptoms in human beings discussed later.

Another pharmacologically important drug demonstrated to interact with DSIP is amphetamine. Pre-injection of 0.1 or 1 mg/kg of DSIP reversed the normal increase in temperature induced by amphetamine in rats at room temperature [153]. DSIP-pretreated rats showed a drastic decrease of their colonic temperature at 21°C and at 4°C, the decrease after 0.1 mg DSIP/kg alone being significant. In addition, most rats treated with DSIP apparently showed sleep-like behavior at room temperature when injected before but not after amphetamine [153]. We could not repeat these results in rats; however, we did find somewhat similar effects in mice [37]. Injection of DSIP, 30 min before amphetamine (15 mg/kg IP), significantly reduced the increase in body temperature in mice. A bell-shaped dose-response curve with an optimal effect at 100–200 nmol/kg IP was found, but 0.1 nmol/kg showed an equal potency. Thus, two different, active dose-ranges of the same peptide were detected in the same model, an unusual finding until now [23,29]. At the same time, D-Ala^4-DSIP revealed an optimal dose-range between 50 and 150 nmol/kg whereas DSIP-P was inactive under the same conditions. Additionally, a seasonal influence on the dose and the effect is possible.

An interaction of DSIP with amphetamine was found in mice also with respect to their locomotor behavior [41]. The increase in activity after injection of amphetamine (10 mg/kg) was significantly more reduced by 120 nmol/kg DSIP (IP) than by 30 nmol/kg. Furthermore, an indication for a biphasic effect of DSIP was found with 15 mg/kg amphetamine. DSIP, at a dose of 30 nmol, first increased the enhanced motor activity of the mice for about 120 min whereas during the same time 120 nmol DSIP/kg IP reduced this activity. Compared to 30 nmol/kg, which was not different from controls, the higher dose increased the locomotor activity significantly at 150 and 165 min, perhaps as a rebound phenomenon to the suppressing effect during the first 2 hr [41].

DSIP alone, in another study, was found to reduce locomotor activity in mice when injected ICV at concentrations of 2.5 to 40 μg/kg [110]. It was also reported that the sleep induced by sodium pentobarbital was prolonged when the mice were injected ICV with DSIP after the barbiturate.

Although the mechanism of DSIP is still obscure, it is possible that at least some of its effects are mediated through serotonergic pathways. Yehuda reported that sleep-responses were induced by combined treatment with brevital and were even greater when the animals were pretreated with either α-methylparatyrosine or l-tryptophan [154]. These effects could be blocked by methysergide, a serotonergic antagonist. Serotonin has long been considered as the major sleep-neurotransmitter [50, 67].

NEUROPHARMACOLOGY, BIOCHEMISTRY, ENDOCRINOLOGY

The sleep-promoting properties by which DSIP was discovered may be related to circadian rhythms. The observation that the effects of DSIP may involve circadian patterns [34] led to further investigations of this influence [32]. In a series of experiments, the effects of DSIP on the levels of the brain neurotransmitters norepinephrine, dopamine, and serotonin were determined in rats in relation to their circadian changes [31]. Depending on the time of injection, evening or morning, different changes in the amount of serotonin and, less prominently, norepinephrine were observed. Serotonin was most markedly reduced during the daytime, especially after an injection of DSIP the previous evening (16 to 20 hr earlier).

At the same time, changes in the percent distribution of plasma proteins were found [31]. It is not known if this represents a real difference in the synthesis and/or degradation of these blood constituents. It is possible that the apparent binding attributes of DSIP to larger proteins in plasma [55] may be involved.

A direct influence of DSIP on enzymes was claimed by Ashmarin and Dovedova [1] when they reported that DSIP increased monoamine oxidase A activity in brain mitochondria. A concentration of 10^{-6} M to 10^{-5} M DSIP increased MAO-A (serotonin substrate) activity in these structures by several hundred percent, whereas MAO-B and acetylcholinesterase were not affected. These observations seem to be in agreement with the reduced serotonin level observed after DSIP injections in rats [31]. Correspondingly, the reversal of the activity of rats injected in the evening [34] would fit with these findings of increased activity the next day with a concomitant decrease of 5-hydroxytryptamine in the brain. DSIP also seemed to induce the activity of adenylate cyclase in the presence of dopamine [71]. Though the effect did not reach statistical significance, it seemed stronger than that of the other peptides tested (except VIP) even at the high concentration used.

In 1981, evidence was presented for specific binding sites of ^{3}H-DSIP in membrane fractions of the pineal gland [38]. Recently, it was found by autoradiography that ^{3}H-DSIP specifically labeled binding sites on neurons but not glial cells of cultured rat brainstem [43]. Thus, brainstem neurons might possess receptors for DSIP, a suggestion consistent with the finding of excitatory effects of DSIP in the nucleus reticularis gigantocellularis [105].

Other activities of DSIP concern influences on hormonal levels. In preliminary experiments, the concentrations of growth hormone (GH), corticosterone, and prolactin in rat plasma were determined. No clear effect of 30 nmol/kg DSIP on the level of GH was observed, which is in contrast to Takahashi *et al.* who found a GH peak 2 hr after IV injection of DSIP in the sleep-deprived dog [145]. However, a reduced concentration of corticosterone was found in the evening when DSIP was injected IV in rats 4 hr before the measurement. Significant suppression of prolactin release over the next 20 hours was observed after either evening or morning injection (unpublished data with J. B. Baumann and G. A. Schoenenberger). It is not known whether these effects of DSIP are mediated through the pituitary gland, but they coincide with the reduced level of stress-response in animals mentioned earlier, and increased resistance against stress in human beings described below.

DSIP EFFECTS IN HUMANS

Perhaps the most intriguing actions of DSIP have been found with human beings. In all human trials, involving a total of more than 70 subjects, no adverse side effects (cardiovascular, respiratory, metabolic) of DSIP have been observed. Beside some symptoms like headache [30] and some other particular reactions (vomiting, vagal reaction, hypotension, discomfort and arousal: one case each) of addicts being withdrawn from alcohol and opiates [17], DSIP was always well tolerated during administration [124].

Several experiments have been conducted by Schneider-Helmert and co-workers to evaluate the sleep-inducing effects of DSIP in man. These researchers applied a broad variety of psychological as well as physiological examinations and performed the experiments under double-blind conditions with placebo controls. The polygraphic recordings of the EEG were analyzed according to the standardized criteria of Rechtschaffen and Kales [115].

Single injections of 25 nmol/kg DSIP into 6 normal subjects at 9 hr in the morning increased total sleep for the next two hours (59% median increase). More striking, however, was the enhancement of sleep during the following night when an increase in slow wave and in REM sleep was found, as determined by t-test with the significance level at $p<0.1$ [124]. With 3 of the same subjects, there was an apparent influence of the duration of infusion; a parabolic bell-shaped curve seemed to occur with the largest effects seen after infusions lasting for 4 to 10 minutes [125].

In another series of sleep experiments, DSIP was injected into 6 patients with severe chronic insomnia immediately before turning off the lights [126,127]. DSIP showed no somnogenic effect during the first hour after injection, but other effects on sleep such as reduced percentage of sleep stage I, less arousal, and more total sleep, were significant (t-test) though not dramatic from the 2nd through the 6th hour [127]. A related finding was reported by Blois *et al.* [8] who described a similar latency of sleep-inducing actions of DSIP in normal subjects. Delayed onset of sleep was also observed in rabbits [95]. In general, DSIP seemed to improve those parameters of sleep that are typically impaired in insomnia.

In further experiments, consecutive treatment over 4 days was applied one hour before bedtime to 4 insomniac patients [129] after laboratory adaptation for one night and placebo injections for two nights. Repeated administration of DSIP increased the sleep-duration in a stepwise manner. Objective measurements (EEG, cardiovascular and respiratory parameters) as well as subjective ratings (visual analog scales, number of awakenings) of the patients showed improvement of severely disturbed sleep to a normal sleep-pattern as evaluated by the Mann-Whitney U test. At the same time, there were indications that the body remained fully reactive to environmental physical stimuli under DSIP. The authors claimed a complete normalization of disturbed sleep of the 4 insomniacs, attributing the effect to treatment with DSIP [128, 129, 139].

Another intriguing observation was the activity of DSIP on the psychophysiology of the waking state in two neurotic patients. One would expect that the sleep-inducing peptide would reduce alertness, but the opposite was found on several occasions and, surprisingly, at the same time when it displayed somnogenic properties [129,139]. After repeated DSIP injections two therapists independently recorded distinct changes which consisted of the patients' feeling more energetic, self-assured, and better relaxed. Although these results were obtained in a specially designed study with only two neurotic patients, similar findings were observed with other subjects treated with DSIP [129,139]. The immediate and delayed somnogenic, but also activating effects are said to depend on whether the external and inner conditions of the subjects support the respective behavior. The most predominant effect of DSIP on sleep in human beings, however, appears to be an accentuation of REM sleep and sleep cyclicity [129].

These studies indicate that DSIP seems to exert an improving or at least sustaining effect on normal and disturbed sleep in human beings. Although the results obtained so far look promising, they are based on experiments that are preliminary in nature, so that definitive conclusions are premature [123]. Additional clinical evaluation is needed to establish more firmly the actions of DSIP on human sleep and perhaps central nervous system disorders.

During the evaluation of the somnogenic and psychotropic effects of DSIP in men, a 69-year-old male being withdrawn from benzodiazepines and antidepressants was treated with DSIP. His sleep pattern, which was completely disorganized, returned to normal after the second injection

of the peptide [139]. A more detailed study designed for the treatment of withdrawal symptoms was reported by a different group at the CINP-Congress in 1982 [16]. Evaluation of treatment was done by observation of the changes in the objective signs of withdrawal noted by a staff member, but no statistics or control groups were used for a more rigid analysis of the results. DSIP-injections as the only treatment produced beneficial effects in 48 of 49 patients (22 alcoholics and 26 of 27 opiate addicts) [17]. The immediate onset of action was primarily a marked suspension of the somatic symptoms whereas anxiety was resolved within hours. The authors proposed DSIP as a new physiologically based approach for the treatment of withdrawal syndromes [17].

HYPOTHESES

A few years ago, we published a short review about DSIP questioning whether this peptide may represent more than just a sleep-peptide [62]. Further considerations about a "sleep-substance" were raised by Borbely and Tobler who proposed 6 requirements for such a compound [10]. According to this, DSIP did not meet the criteria of a sleep-substance because (a) it did not show a distinct and reliable induction and/or maintenance of physiological sleep, (b) no increasing dose-effect relationship was known, (c) the substance showed different sleep effects in different species, (d) changes in the vigilance level were not shown to be associated with alterations in the endogenous concentration of the peptide. Only two criteria, (e) the occurrence in the organism and (f) the chemical identification were apparently accepted by these authors. So far, DSIP (a) has been shown by several groups to induce sleep changes. Point (b) deals with the now widely accepted U-shaped dose-response curve, and point (c) may represent too arbitrary a restriction for the effects of a sleep-substance. We think that points (e) and (f) require further evaluation, and point (d) awaits clarification. Although we do not consider the aforementioned list as an appropriate definition for a sleep-substance, DSIP may fulfill many of the required points.

A major problem with this peptide is the inconsistency of the results. A large variety of models and actions have been tested, but none of the effects has been thoroughly explored in detail. For instance, it remains to be clarified why DSIP increased delta-sleep in the rabbit, whereas in the cat REM sleep was relatively more enhanced. Cats, like most carnivores [25], have a high amount of PS [142] compared to rabbits in which REM sleep accounts for only 5–10% of total sleep [140]. DSIP, therefore, may sustain or enhance that sleep form most relevant to a particular species. One can only speculate what name the peptide would have been given if another species had been chosen to serve as the assay system. Thus, DSIP represents a typical example of the dangers in the naming of natural compounds [54].

However, as discussed earlier [62], DSIP may represent more than a sleep-peptide. It may be too simple to consider insomnia as just a lack of a single sleep-substance. More complex mechanisms are probably involved. The same may be true for withdrawal syndromes. It is not easy to understand how only a sleep substance could have such widespread effects on physiological and psychological systems that also include protection against stress. DSIP seems to act on a different level from that of neurotransmitters. It appears to affect many kinds of reactions in the body, yet a clear or distinct effect on a specific mechanism is lacking. Although hypotheses for DSIP being a "programmer" [138] or "organizing mediator of neurotransmitters" [135], a "programmer" of circadian rhythms [33, 39, 40], a stress-reducer [119,143], or a morphine agonist [146] may eventually be proven correct, none of these is yet established.

All of the hypotheses may involve a modulating action of DSIP on certain neurotransmitter receptors, the action appearing positive or negative depending on the concentration of DSIP that can display several effective peaks. In addition, the endogenous levels of DSIP could fluctuate over time, diurnally as well as seasonally, producing a complex pattern of small effects throughout the body that could eventually result in dramatic changes. Such influences could be more obvious under disturbed conditions like insomnia, withdrawal problems, or psychiatric disorders.

It is also possible that DSIP could influence hormones, including hypothalamic releasing factors, to modulate their actions at the receptor level. Such an interaction could affect a broad variety of somatic events and at a high level of coordination. This would easily accommodate the apparent stress-reducing actions of DSIP. All such speculations may have to be modified if it is found that DSIP is not the physiological, natural peptide but only a fragment of the endogenous compound broken down during its isolation. Therefore, the main questions about DSIP still remain to be answered.

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