Effects of Some DSIP Peptide Analogs on Rat Sleep for Intraventricular Infusion

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Peptide [D-Ala²]DSIP markedly increases the representation of NREM and REM sleep delayed by several hours from the onset of infusion and lasting after the infusion till the end of the 12-hour night period in a chamber. Peptide [D-Tyr¹]DSIP increases NREM sleep during hours IV and XI of recordings. Analogs of these peptides have no noticeable effects.

Key Words: *DSIP peptide; sleep; rats*

Peptide DSIP (Delta Sleep-Inducing Peptide) was isolated from rabbit venous cerebral blood by a group of Swiss scientists in 1977. Intensive investigation of this substance undertaken later by different research teams revealed that DSIP or some other peptide(s) similar in structure is present, free or bound, in the peripheral organs, tissues, and fluids of the body, as well as in the pituitary, hypothalamus, and limbic system of the brain, where it is localized together with a number of peptide and nonpeptide transmitters [2,6,8,14]. There are data on the participation of this peptide in quite a number of normal and abnormal reactions (stress, immune, pain, endocrine), as well as in the regulation of the circadian rhythms, the formation of alcohol and opioid dependence, etc. [2,6,9].

Paradoxically, the "primary" function of this substance - somniferous - is doubted by many workers, and a number of experiments on rabbits and rats, including our own, have not confirmed it [2,5]. However, later we demonstrated that if the N-terminal tryptophan residue were replaced by its D-isomer or tyrosine D-isomer or if the alanine residue in the second position were replaced by its D-isomer in the DSIP molecule (such alterations greatly improve molecular resistance to the destructive action of aminopeptidases), the analogs developed a capacity to prolong NREM and REM sleep in rabbits and rats for intraventricular injection [2-4]. This agrees with the results of *in vivo* and *in vitro* biochemical studies which showed that a DSIP molecule introduced from the outside rapidly disintegrated under the effect of specific amino peptidase and its half-life in the body was just a few minutes [2,6,9]. After the splitting off of the tryptophan N-terminal residue, the horseshoe coiled conformation assumed by the DSIP molecule in aqueous solutions breaks down, this leading to inactivation of the peptide [12].

We studied the effects on rat sleep of 4 DSIP analogs characterized by increased resistance to aminopeptidases: [D-Trp¹]DSP, [D-Ala²]DSIP, and [D-Tyr¹,D-Ala²]DSIP administered by a more "physiological" route, i.e., a slow intraventricular infusion.

MATERIALS AND METHODS

Adult male inbred rats with preimplanted (under barbituric narcosis, 45 mg/kg intraperitoneally) ele-

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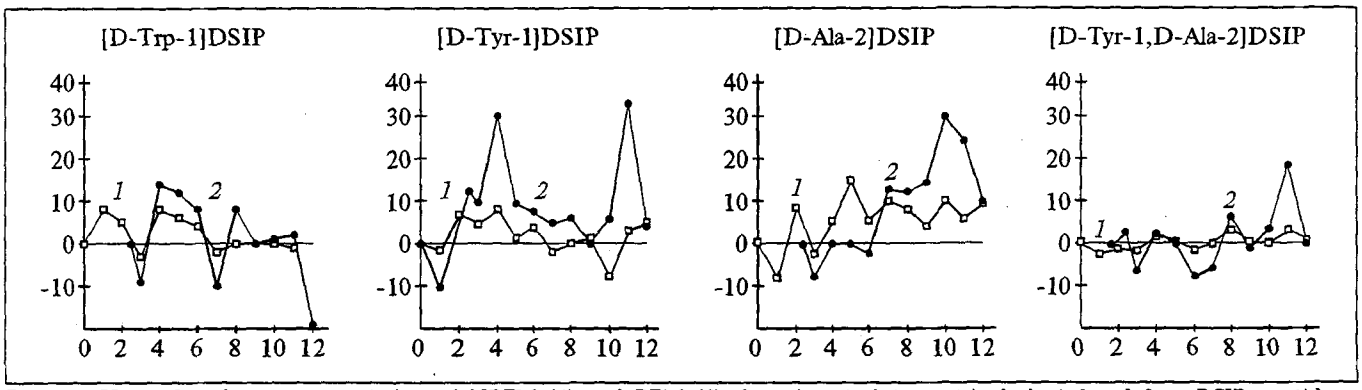

Fig. 1. Hour–by–hour representation of NREM (*1*) and REM (*2*) sleep in rats intraventricularly infused four DSIP peptide analogs in comparison with control infusion of normal saline to the same animals. Abscissa: night period of sleep recording, hours. Ordinate: difference between peptide and control, %. Horizontal line above the diagram marks the 10–hour period of peptide infusion which started one hour before the light in the chamber was switched off.

ctrodes for ECoG and EMG were used in experiments. In addition, a cannula was implanted into each animal (stereotaxically, x-ray monitored) into the third ventricle of the brain. The animals were kept under conditions of unrestricted behavior in an experimental chamber with an artificial climate and were constantly connected to an outlet cable and flexible catheter for infusion. The light in the chamber was switched off at 20:00 h and switched on at 8:00 h. A week after the procedure, constant infusions of normal saline into the brain ventricles were started, using micropumps, at a rate of 20 µl/h, close to the natural production of the CSF. Several days later when the animals had adapted to the infusion, three-day polygraphic recording was started using the following scheme: day 1, control recording; on day 2 one hour before the onset of the dark period in the chamber, that is, at 19:00 h, the saline was replaced with a solution of test peptide in a concentration of 2.5 nmoles in 0.2 ml, which was infused for ten hours, that is, until 05:00 h, after which saline infusion was continued; day 3, recording of aftereffects and recovery. Polygrams were analyzed visually, using routine criteria with a special sensor device hooked up to a PC. The percent representation of both sleep phases during every hour of the record was assessed and the first-day records were compared to those of the second and third days. For statistical analysis Student's bilateral paired test was used.

RESULTS

Figure 1 shows that two of the four tested peptides induced different effects in the nocturnal 12-hour period of the second day of recording in comparison with the first day (control), whereas the other two peptides had no effect on rat sleep. Analog [D-Ala^2]DSIP increased REM sleep starting from the fourth hour till the end of the nocturnal recording period, this increase being reliable during the 5th and 8th hours ($p<0.05\%$, $n=4$), and increased NREM sleep during the second half of the night (reliably during the 11th hour, $p<0.01$). On the whole, NREM sleep increased by approximately 1/3 ($p<0.05$) and REM sleep by 2-fold ($0.05<p<0.1$) over the 12 hours due to a higher incidence (but not a prolongation) of individual episodes. The hypnogenic effect of another analog , [D-Tyr^1]DSIP, was different. This peptide caused a reliable increase of only NREM sleep during the 5th and 11th hours of recording ($p<0.05$, $n=4$). On the whole, NREM sleep increased by 30% (the trend being $0.05<p<0.1$), whereas REM sleep increased by 40% (unreliably) over the 12 hours.

In the following 12-hour day period and on day 3 of recording (not shown in Fig. 1) no changes in sleep were observed as compared to day 1 (control).

What is the possible mechanism of the effect of DSIP peptide analogs on sleep? The long latent period is noteworthy: the effects manifest themselves only several hours after the onset of infusion and last after its cessation. This makes one think that the hypnogenic effect of these peptides may be mediated in some way. Endogenous peptide DSIP has, in fact, been shown to contribute to the regulation of quite a number of peptide hormones, suppressing thyrotropin, corticotropin, and somatostatin release and stimulating luteinizing hormone, somatotropin RF, and growth hormone release [6,10,11,13].

At the same time somatotropin, somatostatin, and, especially, somatotropin RF are capable of increasing REM and, partly, NREM sleep, whereas thyrotropin and corticotropin are known to suppress sleep [7,9]. Hence, metabolically stable DSIP analogs may mediate their effects on sleep via

modulation of the release of the above-named hormones by simulating the releasing/inhibiting function of endogenous DSIP. If this hypothesis is confirmed it will be one more example of a cascade type of peptidergic regulation in "multipurpose" neuronal systems [1].

But if this is so, what is the cause of the absence of activity in two of the tested analogs? Since all four peptides are characterized by increased resistance to aminopeptidases, differences are possible in the rates of degradation under the effect of other brain enzymes (carboxypeptidases and endopeptidases). Studies of the effect of [D-Trp^1]DSIP peptide on sleep have shown its activity for a relatively rapid (by injection) intraventricular administration to rabbits and rats [2-4]; however, no manifest effect was observed for a 10-hour infusion to rats in any of the hours of recording ($p>0.1$; $n=5$). A possible explanation for this phenomenon is provided by a study [15] showing that angiotensins are more effective with respect to the drinking behavior of rats when injected intraventricularly than when they are infused; thus, the hypothesis is that there is a gradual increase of the activities of proteolytic enzymes during slow administration of peptides, and therefore the delivery and degradation of the peptides are counterbalanced. In contrast to this, during rapid administration the peptidases fail to decompose the whole aliquot, and at least part of it manages to enter into interaction with a receptor or defense protein. Hence, a more "physiological" method of administration may sometimes be less effective.

As for the absence of an effect on sleep on the part of a disubstituted analog [D-Tyr^1,D-Ala^2]DSIP ($p>0.1$, $n=7$), the most probable explanation is conformational disorders rendering interactions between the analog molecule and the receptor impossible. The same specificities of conformational interaction appear to be responsible for the differences in the hypnogenic effects of peptides [D-Tyr^1]DSIP and [D-Ala^2]DSIP.

Hence, these data confirm our previous results obtained in other test systems (intraventricular injections to rabbits and rats [2-4]) that an inactive DSIP molecule acquires hypnogenic characteristics if its proteolytic stability is increased and optimal conformation preserved. These characteristics are expressed in a higher probability of sleep development during certain periods of time, that is, they are of a modulatory nature, in contrast to the effects of barbiturates and benzodiazepines. The ability of DSIP analogs to cross the blood-brain barrier for systemic administration [2,3,6,9] makes them suitable candidates for clinical application.

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