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Cortical Administration of Somatostatin (SRIF): Effect on Sleep and Motor Behavior[1]

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REZEK, M., V. HAVLICEK, K. R. HUGHES AND H. FRIESEN. *Cortical administration of somatostatin (SRIF): effect on sleep and motor behavior.* PHARMAC. BIOCHEM. BEHAV. 5(1) 73–77, 1976. – Cortical administration of SRIF in unrestrained, freely moving rats produced an early activation, stereotyped behavior patterns and later, coordination difficulties often associated with drowsiness. A few animals showed a tendency toward paraplegia-in-extension. A considerable, prolonged alteration in the sleep-waking cycle was also observed. Similar results were obtained in both intact and hypophysectomized animals. Intraperitoneal administration of SRIF induced several other effects in addition to those seen after cortical application. The latter were however, restricted in variety, intensity and duration.

Somatostatin (SRIF) Sleep Motor behavior Intact animals Hypophysectomized animals
Cortical administration Intraperitoneal administration

THE EXPERIMENTAL and clinical reports on systemic and cerebroventricular administration of hypothalamic releasing and inhibiting hormones have yielded new evidence which strongly indicates that these substances might exert a direct nonendocrine action in the CNS [5, 7, 9, 10]. Of the increasing number of newly identified hypothalamic hormones SRIF and thyrotropin releasing hormones (TRH) have recently attracted our attention and are now systematically investigated in our laboratory. The cerebroventricular as well as direct cerebral tissue administration of these hormones consistently induced a variety of motor, behavioral, electrophysiological and biochemical changes [4,12]. These experiments also involved a study of the dose-response relation of SRIF action as well as its specificty in the CNS by the use of biologically active and inactive analogues of SRIF [12].

The present report analyzes the effects of cortical administration of SRIF and its long term influence on the sleep-waking cycle. This investigation was stimulated by differences in the pattern of motor and electrophysiological changes observed in several animals whose intended intraventricular cannulas were later shown to have been positioned in the cortex dorsolaterally from the ventricle. For this reason we studied this phenomenon in more detail by applying SRIF directly on the cortical surface at the same dose and at the same anterodorsal and lateral coordinates as our previous ventricular infusions. Since our pilot study indicated rather prolonged alterations of the sleep-waking cycle, we decided to incorporate into our design several observations and recordings made at various times after the initial administration of SRIF. For a better assessment of the central effects of SRIF induced by systemic and direct cerebral administration of this hormone, SRIF was also administered intraperitoneally via chronic cannulas under the same experimental conditions as those for animals receiving cortical infusions.

METHOD

Male Sprague-Dawley rats were used in the present experiment. For cortical administration of SRIF – 7 intact of 6 hypophysectomized animals were implanted with a chronic supracortical cannula [11] (DeGroot stereotaxic coordinates: A = 3.2, L = 5.0) along with four epidural electrodes positioned bilaterally over the sensorimotor cortex (bipolar recordings, electrode separation 3 mm).

After the surgery animals were allowed to recover and were habituated to experimental procedure, this being done by the transfer of the animals into the recording chamber, insertion of the infusion cannula and EEG connector with subsequent stay of the animal in the recording chamber for 2 hr. The experiment itself was performed in an electrically shielded room equipped with facilities for the recording of the electrical activity of the brain, a one-way mirror and the apparatus allowing remotely controlled infusions of experimental substances. The recording chamber had a gridfloor to allow for a better detection of changes in the fine motor coordination. With the aid of a piezo-electric system even the minor vibrations of the cage grid floor were recorded.

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After the habituation to the experimental procedure, the animals were placed into the recording chamber, connected to the infusion pump, EEG polygraph and, 15 min later, were given SRIF supracortically in a dose of 10 μg at a rate of 1 μg/μl/min. Before any SRIF infusion was performed, all animals were infused with 10 μl of control CSF [2] at the same infusion speed. During and for 2 hr after the end of infusion the behavioral and motor manifestations were monitored continuously through a one-way mirror For the same period the electrical cortical activity was recorded on polygraph and magnetic tape. The polygraph recordings were used to determine the various states of the animal (awake, SWS, REM sleep) as a basis for the analysis of alterations in the sleep-waking cycle. Tape recordings were to be used for a subsequent Fast Fourier analysis of frequency spectrum [3]. To assess and quantify the changes in muscle tone and reactivity by handling and tactile stimulation we have used a scoring scale from 1–5 in the following manner: (1) complete muscle flaccidity and no response to tactile stimulation; (2) slight resistance in the muscles of the hind limbs to flexion and extension or slight resistance of the tail to lateral manipulation and detectable response to tactile stimulation; (3) moderate resistance and response; (4) marked resistance and response; and (5) muscle spasticity or hyperreactivity to tactile stimulation. Each animal was later infused with CSF once again and monitored for 2 hr once during the first 14 days after the administration of SRIF. The same procedure was repeated during the second 14 days to estimate the recovery from a variety of motor, electrophysiological and sleep-waking alterations induced by the previous administration of SRIF.

Four animals with chronic intraperitoneal cannulas and epidural electrodes were habituated to the experimental procedure in the same manner as animals with cortical cnnulas. Subsequently they were administered SRIF in the dose of 100 μg prepared in 5 ml of physiological saline and infused over the period of 15 min. As a control administration, 5 ml of physiological saline was infused over the same period before any administration of SRIF was performed. The same variables as those monitored following cortical infusions were recorded and analyzed during and for 2 hr after the intraperitoneal infusions of control saline and SRIF. The alterations in sleep-waking cycle were assessed statistically by comparing the duration of periods of sleep and wakefulness by means of an analysis of variance and Duncan's multiple range test.

RESULTS

The cortical administration of SRIF produced behavioral and electrophysiological changes which were in some respects similar to those induced by previous intraventricular administration of SRIF [4]. On the other hand, several other manifestations were distinctly different. For example, early after the beginning of infusions the animals displayed signs of activation consisting of sniffing and horizontal exploration which was often associated with repeated cleaning and scratching movements corresponding to Stage 1 on our behavioral scoring sclae [4]. Also, many examples of behavioral stereotypy (Stage 2A) were detected (some or all of the following: freezing, tremor, quiver of the lower jaw, biting the floor bars, chewing, licking, repeated generalized shaking, circular movements) but these were generally less intense and shorter in duration than similar manifestations previously observed after the intraventricular infusions. On the other hand, difficulties in motor coordination (poor finger grip, slipping of the legs, loss of balance) developing during or shortly after the end of infusions were more apparent or prolonged following cortical infusions in the present experiment (Stage 2B). Frequently these difficulties became more severe so that animals lay motionless with legs extended between the floor bars. Occasionally, they were gasping with eyes wide open and nose suspended between the floor bars. Infrequent attempts to pull the legs up were crude, uncoordinated and often unsuccessful as the legs repeatedly slipped between the bars. When touched, the animals appeared stiff but were not hyperreactive to tactile stimulation as previously observed in the animals given SRIF intraventricularly. This is reflected in the results of our tests designed to assess the changes in muscle tone and reactivity to external stimulation (Table 1) which revealed only an increase in rigidity scores when recorded at the end of a 2 hr postinfusion test period.

In 20% of the animals a tendency toward paraplegia-in-extension (Stage 3) was detected as animals were either lying on one side or had a tendency to tilt to one side due to the predominant extension of contralateral extremities.

During the period of motor coordination difficulties, the electrical activity of the cortex underwent changes characterized by a reduction in amplitude and sometimes associated with a reduction of background activity. Although these electrophysiological changes were not of the same magnitude in all animals, the amplitude of the

TABLE 1

EFFECT OF SRIF ON REACTIVITY AND RIGIDITY AS RECORDED BEFORE AND IN THE END OF A TEST PERIOD FOLLOWING THE ADMINISTRATION OF CSF AND SRIF (SCORING SCALE FROM 1 TO 5)

	Reactivity			Rigidity		
	Scratching on the back	Touching the whiskers	Air blown on the face	Lifting the animal from the grid floor	Flexion and extension of the back paw	Lateral movements of the tail
Pre-CSF	2.0	2.8	2.8	2.2	2.0	1.6
Post-CSF	1.8	2.2	2.6	2.2	1.6	1.2
Pre-SRIF	2.1	3.4	2.6	2.2	1.5	1.5
Post-SRIF	1.9	3.1	2.4	2.5	2.6*	2.6†

Statistically significant results at 0.05 and 0.01 levels are indicated by * and † respectively.

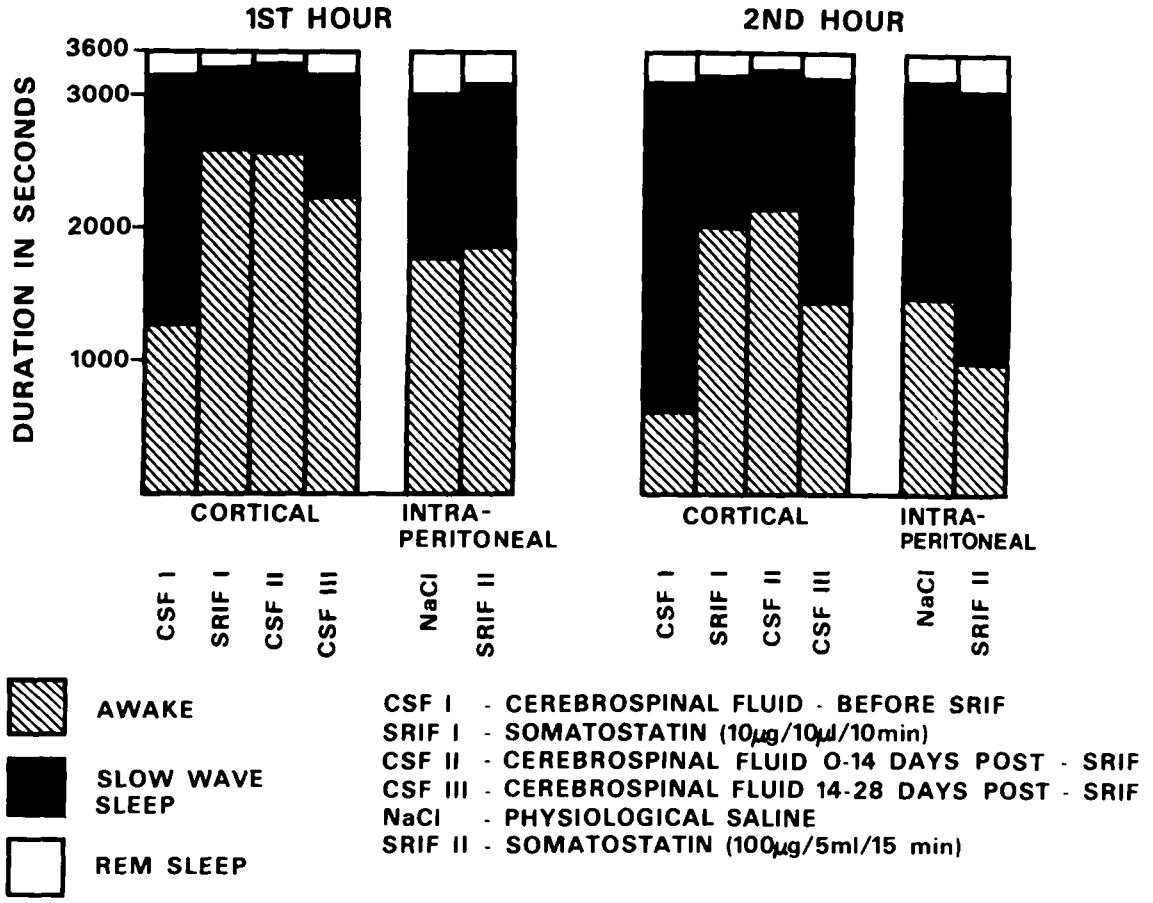

FIG. 1. The duration of various periods of sleep-waking cycle following cortical and intraperitoneal administration of SRIF.

electrocorticogram was reduced in several animals and in one case temporarily flattened to the extent that it resembled the activity during the phenomenon of spreading depression. This pattern was infrequently interrupted by brief periods of hypersynchronized slow wave high voltage electrical activity (more typically observed after previous intraventricular infusions) especially in animals displaying a tendency toward paraplegia-in-extension.

Also, the sleep-waking cycle showed a marked alteration for a prolonged period of time. Thus, a significant reduction in the duration of both the slow-wave sleep $F(3,48) = 8.27$, $p<0.01$ and REM sleep $F(3,48) = 14.98$, $p<0.01$ was recorded throughout the entire 2 hr test period (Fig. 1) as the duration of the awake state of animals was proportionally prolonged $F(3,48) = 13.70$, $p<0.01$. The reduction in the total sleeping time, was, however, smaller than that previously seen after intraventricular infusions; this being due mainly to a smaller reduction in SWS. The initial stages of SWS were frequently detected during the period of coordination difficulties and the animals generally appeared to be drowsy. The SWS seen in most SRIF-treated animals was somewhat different from controls; it was mainly of shallow and superficial character (drowsiness) with peculiar frequent oscillations of brief periods of SWS and wakefulness (20–50 sec) which rarely progressed into a deeper SWS or REM sleep.

The repeated examination of the sleep-waking cycle during the 1st two weeks post-SRIF showed a prolonged residual effect of SRIF administration: the duration of SWS was not significantly increased and REM sleep was, in fact, further slightly decreased when compared with the corresponding data acquired at the time of SRIF administration.

The second reexamination revealed a trend toward recovery of sleep-waking cycle: total duration of REM sleep during the entire 2 hr test period now practically returned to the pre-SRIF control level and also the total duration of SWS, although still significantly shorter ($p<0.05$) than pre-SRIF control value, showed a considerable increase in comparison with corresponding post-treatment value (Fig. 1). The sleep-waking pattern during the first and to a smaller extent during second reexamination was again often characterized by frequent oscillations of shallow SWS and wakefulness. All behavioral, motor and electrophysiological manifestations were observed in both intact and hypophysectomized animals; since the difference between these groups was not significant the present results represent in all respects the mean of combined data obtained from both intact and hypophysectomized animals.

Several intraperitoneal infusions of SRIF performed in an attempt to compare the effects of central and peripheral administration, induced many of the symptoms described above. Thus an early activation during the infusion was later accompanied by several signs of stereotyped behavior (tremor, chewing) although their variety and duration appeared reduced. However, new manifestations such as repeated stretching of the body and lordosis were noticed.

Coordination difficulties were less severe and only of transient character. The predominant characteristics of the response to tactile examination was that of stiffness. The sleep-waking cycle was also influenced by intraperitoneal infusions but clearly to a much lesser extent (Fig. 1) than by cortical or by the previous intraventricular infusion. For example reduction in REM sleep was found only in the first postinfusion hour with an apparently compensatory increase in SWS and REM sleep during the second hour (Fig. 1) in marked contrast to the prolonged character of sleep deprivation following cortical infusions.

DISCUSSION

The present findings provide further evidence for the concept of a central action of hypothalamic hormones. This is based on the results of direct administration of SRIF in cerebral tissue whereas almost all of the presently available information on this subject is the result of systemic or intraventricular administration of SRIF or other hypothalamic hormones. This may indicate that the natural occurrence of SRIF in the cortex [14] may be of physiological importance in modifying the normal functions of CNS. Although the dose used in the present experiment is relatively high, the primary reason for its use was an attempt to compare its effects with those induced by the same dose of SRIF applied intraventricularly. Furthermore, the rate of our infusions (1 $\mu g/\mu l/min$) was slow enough so that a continuous monitoring during the infusion enabled approximate correlation of developing symptoms with an increasing dose. The dose-response aspect as well as the specificity of SRIF action was studied in detail in our previous experiments [12].

The present results revealed a similarity of the behavioral, motor and electrophysiological manifestations induced by cortical and the previous intraventricular administration of SRIF [4]. The cortical infusions, however, did not induce a complete syndrome of paraplegia-in-extension and in no instance generalized tonic-clonic seizures developed. Also, the various symptoms of stereotyped behavior were not as intense and prolonged. On the other hand, cortically tested animals showed a longer duration of coordination difficulties associated with drowsiness in contrast to a marked and prolonged activation which was not influenced by motor coordination problems in the intraventricularly tested animals. This variation in the intensity and time-course of these manifestations reflect the differential involvement of the motor and association cortex following cortical infusions as opposed to the striatal structures and hippocampus which have the largest surface exposure to the lateral ventricle and are the primary target tissues and possible mediators of effects observed after the intraventricular infusions [4]. It may represent two levels of central motor control and thus can probably be explained on the basis of the mechanism of the pyramidal and extrapyramidal motor systems combined with the role of a suspected limbic pacemaker for stereotyped behavior [6,12].

Of special interest is the character and duration of alterations in the sleep-waking cycle. The overall reduction in sleep was considerable and significant but clearly smaller than after intraventricular infusions. A proportionately greater reduction in REM than in SWS and the frequent oscillations of shallow SWS and wakefulness may suggest that most of the sleep time represents a superficial shallow SWS (animals often sitting or crouching in a position atypical for sleep due to the predominant stiffness of muscles in the extremities) as the animals appeared to be unable to progress into more advanced stages of the sleep. This limited the deprivation primarily to a deeper SWS and REM sleep.

The differences between the effect of the central and peripheral administrations of SRIF such as the reduction in the intensity and duration of behavioral and motor manifestations along with the limitation of sleep-wakefulness alterations to the first postinfusion hour following intraperitoneal administration of SRIF may indicate that the peripheral route of introduction may not provide the best insight into the central mode of action of this hormone. In addition, some of the manifestations accompanying the intraperitoneal administration (stretching, protruding of the belly, lordosis) could have been indicative of nonspecific, peripheral effect [8].

Since little is known regarding the possible mechanism of action of SRIF, it is difficult to speculate on the nature of prolonged effect of SRIF in producing alterations of the sleep-waking cycle. This is certainly of interest in view of rather short biological half-life of SRIF in the brain [1] as well as its brief effect on growth hormone levels in the periphery. Also, we have recently observed that alterations in the cyclic AMP levels in various regions of the brain following the central administration of the same doses of SRIF is of relatively transient character (Havlicek, *et al.*, unpublished observation). Finally, the possibility of conditioning to the experimental situation during the repeated recordings should also be considered. Thus until more relevant evidence is provided it can only be concluded that present results support a concept of central nonendocrine action of endogenous SRIF whose presence has been determined in the extrahypothalamic regions of the brain.

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