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Somatostatin and Thyrotropin Releasing Hormone: Central Effect on Sleep and Motor System[1]

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HAVLICEK, V., M. REZEK AND H. FRIESEN. *Somatostatin and thyrotropin releasing hormone: central effect on sleep and motor system.* PHARMAC. BIOCHEM. BEHAV. 4(4) 455–459, 1976. – The hypothalamic hormones, somatostatin (SRIF or GH-RIH) and thyrotropin releasing hormone (TRH) applied intraventricularly into rat brain had a considerable effect on motor function and resulted in profound alterations in the sleep-waking pattern. While TRH induced primarily an increase in exploratory and motor stereotyped behavior, the effect of somatostatin was strinking and prolonged: stereotyped circular running in many instances evolved into catatonia, paraplegia-in extension and/or tonic-clonic seizures.

Somatostatin Thyrotropin releasing hormone Sleep-waking cycle Motor behavior Central effect

THE SEARCH for hypothalamic factors controlling the function of the adenohypophysis has led to the isolation of at least three peptides whose chemical structure has been established [6, 8, 31]. These factors – thyrotropin releasing hormone (TRH), luteinizing hormone releasing hormone (LHRH) and growth hormone release inhibiting hormone (somatostatin or SRIF or GH-RIH) – were believed to be localized in the hypothalamus and to have very specific effects on pituitary function. Recent evidence which has been obtained by using sensitive assays for the detection of hypothalamic peptides [2,3] indicates, however, that they are distributed widely in the brain not only in mammals [7, 17, 32, 24] but also in submammalian chordates lacking an adenohypophysis [18]. These findings, together with gross behavioral [22, 24, 33], clinical [19,25] and discrete neuronal studies [26,27] suggest that TRH may play a role in neurotransmission within the CNS which is independent of its effect on the adenohypophysis. As a result, the possibility of other hypothalamic hormones having similar effects on the CNS has been raised [20,23].

In the present study, we have examined the central effect of somatostatin in comparison with TRH on motor behaviour and the sleep-waking cycle in normal and hypophysectomized rats.

METHOD

The animals were male Sprague-Dawley rats (200–250 g weight) implanted with a chronic intraventricular cannula [28,29] and platinum epidural electrodes. Three to four weeks recovery was allowed for hypophysectomized animals. Experiments were performed between 9:00 and 16:00 hr in a sound-proof and electrically shielded chamber (20 × 21 × 35 cm) having a grid floor. After habituation to the experimental environment (5 2-hr sessions), animals were infused intraventricularly (1 μl/min, maximum volume 10 μl) with the artificial cerebrospinal fluid alone [10] or with somatostatin (SRIF) or TRH prepared in the same vehicle. There were 5–6 animals in each group. Polygraphic recordings were taken during the infusion and, subsequently, during the two hours following the infusion. Our techniques for the recording EEG, fast Fourier analysis of EEG and polygraph recording of sleep have been described elsewhere [13,15]. Three states of the animals were differentiated on the basis of the EEG recordings: (1) awake, (2) slow wave sleep (SWS) and (3) REM sleep. The motor behavior of an awake freely-moving rat was continuously assessed by visual inspection and marked on the EEG record. Five stages (0–4) of the motor behaviour were differentiated. These stages are described below in our results.

RESULTS

Somatostatin in a dose of 10 μg (1 μg/μl/min) in normal and hypophysectomized animals produced a marked behavioral excitation which resulted in a profound reduction of SWS and REM sleep: the former was reduced more than 5-fold during the first hour and more than 2.5-fold

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during the second hour, while the latter was almost abolished during both periods (Fig. 1). By comparison, TRH in the same dose of 10 μg infused at the same rate (1 μg/μl/min) reduced the SWS and REM sleep only during the first postinfusion hour and, to a lesser degree than after somatostatin treatment (Fig. 1).

An even more remarkable difference between the action of somatostatin and TRH on motor behavior was detected during the waking state of the animals. Five stages of motor behavior were differentiated.

Stage 0. Sitting or lying with open eyes, occasional movements, grooming and/or cleaning.

Stage 1. Exploratory behavior with horizontal and/or vertical movements with dislocation in the cage accompanied by sniffing.

Stage 2A. Some or all of the following symptoms are observed: stereotyped movements or unilateral circular movements, (compulsory scratching, chewing, sniffing) often interrupted by freezing or catatonic reactions, a stiffly arched tail over the animal's back (Fig. 2a) quiver of the lower jaw due to tremor of the m. masseter, generalized body tremor, muscle rigidity.

Stage 2B. The same symptoms as in the Stage 2A accompanied by a disturbance of balance. This symptom is especially apparent on a grid floor since the grip of the floor bars is impaired due to the spasticity in the extensors of the limbs (Fig. 2b).

Stage 3. Paraplegia-in-extension with a complete loss of equilibrium due to the spastic rigidity which predominates especially in the extensors contralateral to the side of infusion (Fig. 2c).

Stage 4. Generalized tonic-clonic seizures (Fig. 2d).

TRH caused only a general arousal reaction, an increase in exploratory behavior and stereotypy which included chewing, licking, sniffing and compulsory scratching (Stages 1 and 2a in Table 1). Somatostatin, on the other hand, induced more intense motor excitation and stereotypy was composed of more prolonged spells of compulsory scratching and circular movements. A very important feature of this initial stage of the somatostain action was the disturbance of balance, gait and motor coordination (Stage 2b); this symptom was absent after TRH treatment (Table 1). The most apparent difference between TRH and somatostatin treatment was noted in those animals which reached the higher stages of somatostatin action. After an initial restlessness and catatonia, 67% of animals developed paraplegia-in-extension with the rat lying on one side due to the spastic rigidity prevailing in the limb extensors contralateral to the side of infusion (Stage 3, Table 1, Fig. 2c). Moreover, 33% of the animals exhibited tonic-clonic seizures (Stage 4, Table 1, Fig. 2d); some animals reached this stage after a brief spell of catatonia while others developed seizures after short episodes of paraplegia-in-extension. More violent contractions of limbs contralateral to the side of infusion caused animals to rotate unidirectionally along the body axis. None of these more advanced stages (Stages 3 and 4) were observed in TRH treated animals (Table 1).

When infused in the same dose (10 μg) at the slower rate (0.5 μg/0.5 μl/min) somatostatin never induced pathological syndromes such as paraplegia-in-extension or seizures (Stages 3 and 4). Sleep was affected to a lesser degree in these experiments than in experiments when somatostatin was infused at a regular rate of 1 μg/μl/min. The following significant differences between the fast and

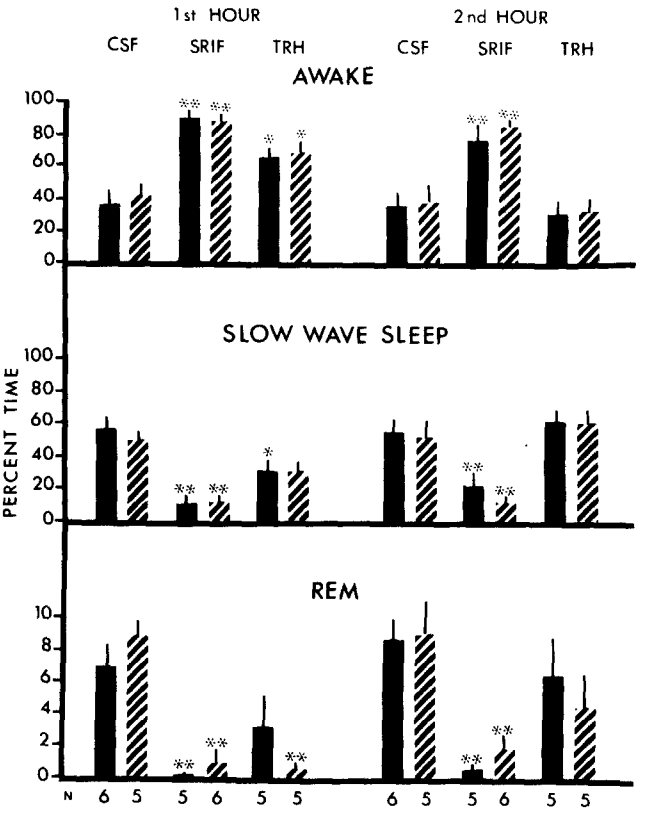

FIG. 1. Effect of the intraventricular infusion of SRIF (10 μg) and TRH (10 μg) on sleep and wakefulness in normal (n = 4) (black bars), hypophysectomized (n = 6) (shaded bars) rats. The duration of the three states – awake, slow wave sleep (SWS) and REM sleep – is expressed in percent of the total measurement during the first (1st hr) and second (2nd hr) postinfusion hour. Infusion of the artificial cerebrospinal fluid (CSF), somatostatin (SRIF) and thyrotropin releasing hormone (TRH) was performed at a rate of 1 μl/1 min. * = $p \leq 0.05$, ** $p \leq 0.01$ (multiple range Duncan test). Differences between normal and hypophysectomized rats are nonsignificant. Data are expressed as Mean ± Standard Error.

the slow infusion were seen during the second postinfusion hour: in comparison with the fast infused animals the duration of the slow wave sleep was two-fold longer in the slow group while the duration of the awake state changed in the opposite direction (Table 2).

DISCUSSION

The present findings, in accord with the recent observations of other investigators [20, 22, 23, 24, 27, 33] suggest that hypothalamic hormones may have a direct central effect which is independent of their well-established action on the adenohypophysis. The behavioral [20, 22, 23, 24] and toxic [5] effects seen after the systemic application of some of these hormones (TRH, LHRH, SRIF) should, however, be interpreted with extreme caution since the free passage of these substances across the blood-brain barrier has not as yet been demonstrated. While in experiments with the systemic application of TRH most authors agree on its psychogenic action [22, 24, 33] results obtained in clinical investigation are controversial: some authors report a pronounced rapid relief of symptoms of depression [19,25] others question these claims [4,21]. Analogous

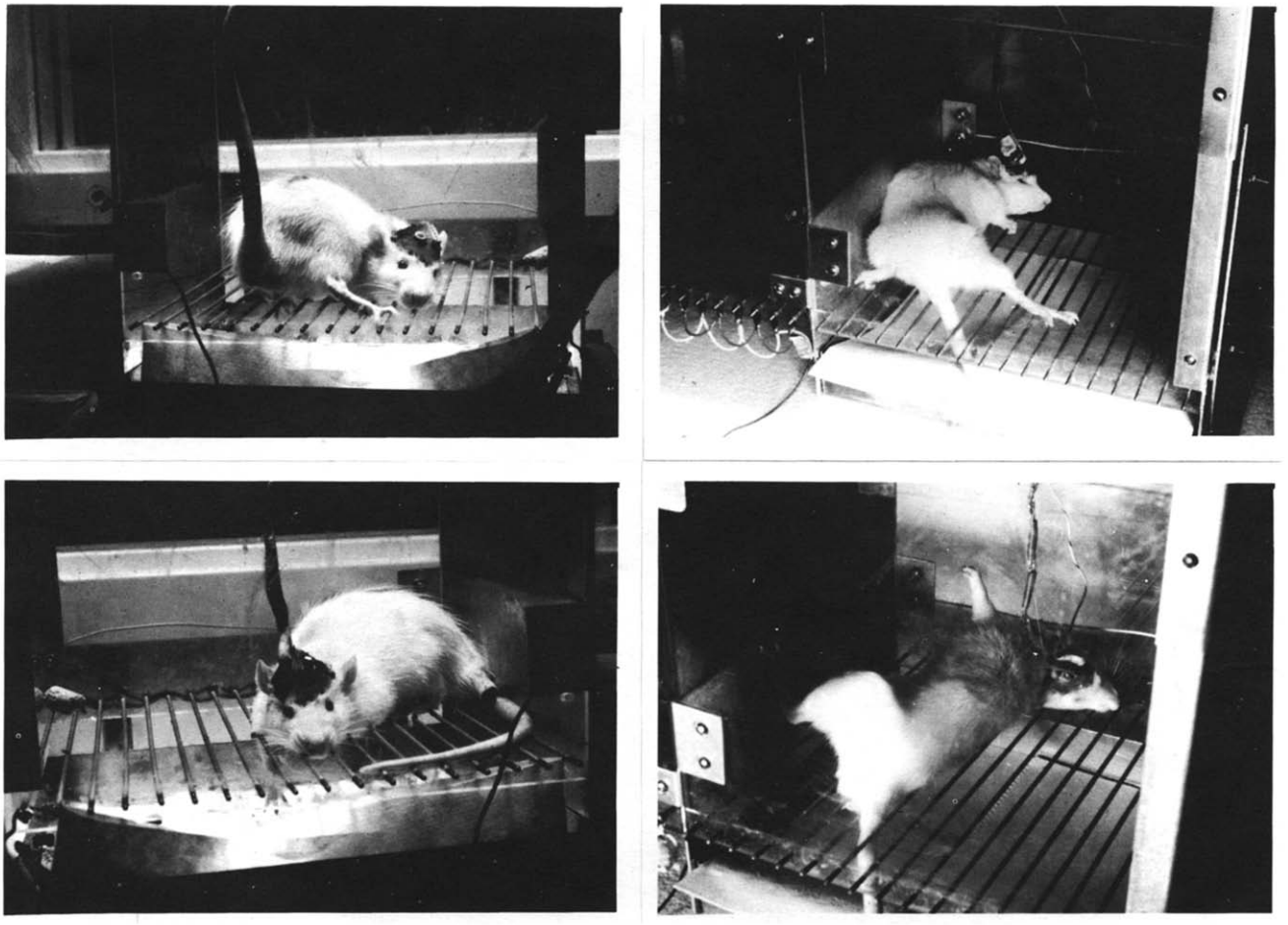

FIG. 2. Effect of somatostatin on motor behavior. Top left: Stiffly arched tail (Straube sign) – Stage 2A. Bottom left: motor difficulties resulting in loss of balance (note front right leg with extended digits hanging between bars of the floor) – Stage 2B. Top right: paraplegia-in-extension – Stage 3. Bottom right: Tonic–clonic seizures – Stage 4.

TABLE 1

EFFECT OF SOMATOSTATIN (SRIF) AND TRH UPON THE MOTOR SYSTEM IN RATS

Treatment	No. of Rats		First Hour Postinfusion STAGE 0	1	2A	2B	3	4	Second Hour Postinfusion STAGE 0	1	2A	2B	3	4
CSF	11	% of Animals*	100	100	0	0	0	0	100	90	0	0	0	0
		% of Time†	29	11	0	0	0	0	23	10	0	0	0	0
		±S.E.	4	3	0	0	0	0	4	3	0	0	0	0
TRH	10	% of Animals	100	100	90	0	0	0	100	80	50	0	0	0
		% of Time	31	20	21	0	0	0	18	15	5	0	0	0
		±S.E.	5	4	4	0	0	0	4	4	3	0	0	0
SRIF	12	% of Animals	33	92	100	100	67	33	73	100	82	27	0	0
		% of Time	14	16	21	31	8	33	28	32	21	2	0	0
		±S.E.	5	3	6	5	4	10	6	7	7	1	0	0

*Data are expressed in percent (%) of animals reaching a certain stage as defined below. The data from normal and hypophysectomized animals were pooled as there was no statistically significant difference in motor response between these two groups.

†The duration in which animals persisted in each stage during the first and second hour after treatment is calculated as a percent of the total duration of the measured period, i.e. one hour. The definition of stages see text.

TABLE 2

EFFECT OF THE INTRAVENTRICULAR INFUSION OF SOMATOSTATIN (SRIF) ON SLEEP AND WAKEFULNESS IN NORMAL RATS*

Treatment	SWS	REM	AWAKE
	First Hour Postinfusion		
CSF (A)	57.0±7.7	7.0±1.4	35.9±8.7
	A:B†	A:B†	A:B†
SRIF (B)	10.3±5.5	0.1±0.1	89.6±5.6
	A:C†	A:C†	A:C†
SRIF (C)	22.3±5.5	0.2±0.2	77.5±5.4
	Second Hour Postinfusion		
CSF (A)	66.7±4.2	8.5±1.3	24.7±4.1
	A:B†	A:B†	A:B†
SRIF (B)	22.3±9.4	0.6±0.3	77.1±9.6
	B:C†	A:C†	B:C†
SRIF (C)	54.2±9.5	2.8±0.3	43.0±9.5

*Somatostatin was applied in the same dose (10 μg) at two rates 1 μg/ μl/min (B) and 0.5 μg/0.5 μl/min (C). The duration of the three states—awake, slow wave sleep (SWS) and REM sleep—is expressed in percent during the first and second postinfusion hour. Control rats were infused with the artificial cerebrospinal fluid (CSF) (A) at the rate of 1 μl/min (10 μl). In each group there were four to five animals. Data are expressed as Mean ± Standard Error.
†$p<0.01$ (multiple range Duncan test).

dissimilarities can be found in the results obtained with systemic application of somatostatin: one group of authors finds that this hormone significantly potentiates the excitatory behavioral effects of DOPA [23], while others describe a reduction of strychnine toxicity and potentiation of pentobarbital toxicity which is interpreted as evidence for a central depressant action of somatostatin [5]. In experiments of this nature it is very difficult to differentiate between the direct CNS effect and the reflex changes in the CNS due to primary peripheral action of these hormones: stimulation of the autonomic nervous system [16], changes in glucose metabolism [11,12] etc.

Central application avoids to a great extent these complications and assures a more direct central action of these hormones. When the effects of TRH and somatostatin are compared in experiments with intraventricular application, the latter is shown to exert a considerably stronger excitatory action than does the former.

Moreover, this potent excitatory effect of somatostatin dramatically exceeds the similar effect of another better known putative neurotransmitter in the brain – glutamic acid. In our experiments performed on the same animals, glutamic acid applied intraventricularly in a dose of 10 μg produced hardly any excitatory effect, while somatostatin, administered in the same dose and via the same route, induced a very strong motor excitation and, in several instances, seizures. A considerably higher dose (approximately 100 times larger) of glutamic acid was needed in order to induce seizures. It is unlikely that pathological symptoms, such as disturbances of gait and equilibrium, paraplegia-in-extension and tonic-clonic seizures seen in our experiments after 10 μg of somatostatin, are due to nonspecific general toxic side-effects of somatostatin. The LD_{50} of somatostatin in rat applied systemically or centrally is not yet known. However no toxic effects were observed in the rat after the infusion of 50 μg intracerebrally [9] nor in mice at a doese of 10 mg/kg intraperitoneally [23]. Our other experiments indicate that different areas of the brain have different sensitivities to somatostatin. Thus, somatostatin applied supracortically in the dose of 10 μg/10 min never induced seizures nor paraplegia-in-extension (Havlicek, Rezek *et al.*, manuscript in preparation. Topical tissue applications of somatostatin to different limbic structures exerted a dose-dependent stimulatory effect, in some instances inducing general arousal reaction, in others a specific behavioral pattern; a distinct awakening effect has been observed at a dose as low as 0.01 μg [30]. Moreover, the present experiments show that pathological CNS symptoms occuring after 10 μg of somatostatin can be avoided if the rate of intraventricular infusion is reduced from 1 μg/min to 0.5 μg/min (Table 2). This finding also indicates that in order to produce pathological CNS symptoms somatostatin must reach a critical concentation in certain areas of the brain. During a slow infusion this concentration was not reached due to several possible mechanisms such as passive removal with the flow of the cerebrospinal fluid, metabolic deactivation or uptake mechanisms. Which of these is the primary mechanism of somatostatin deactivation is an intriguing question. The status of somatostatin as a bona fide CNS neurotransmitter depends on answers to these questions.

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