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Changes in Sleep in Response to Intracerebral Injection of Insulin-Like Growth Factor-1 (IGF-1) in the Rat

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Changes in sleep were studied during 6 hours after intracerebroventricular (ICV) administration of Insulin-like growth factor-1 (IGF-1) or the structurally related insulin. IGF-1 was injected either at dark onset (0.05 or 0.5 µg) or 6 hours after light onset (0.05, 0.5, or 5.0 µg). The small dose of IGF-1 consistently, albeit modestly, enhanced NREMS over the 6 hour postinjection period in both the dark and the light cycles (REMS increased only at night). The NREMS-promoting activity vanished when the dose was increased to 0.5 µg, and 5.0 µg IGF-1 elicited a marked and prompt suppression in NREMS. Heat-inactivated IGF-1 (0.05 µg) did not alter sleep. On a molar base, the NREMS-promoting dose of insulin was higher than that of IGF-1. Late (hours 7-17 postinjection) enhancements in EEG slow wave activity during NREMS were observed after 5.0 µg IGF-1. The results indicate that IGF-1 can promote NREMS and may contribute to the mediation of the effects of GH on sleep. The acute sleep-suppressive activity of the high dose of IGF-1 is attributed to an inhibition of endogenous growth hormone-releasing hormone (GHRH).

CURRENT CLAIM: IGF-1 may contribute to the mediation of the effects of the somatotropic axis on sleep.

Insulin-like growth factor-1 is a hormone secreted by the liver, and an autocrine/paracrine substance produced in various tissues, including the brain. IGF-1 is structurally related to IGF-2 and insulin. IGF-1 has a low affinity to insulin receptors and a high affinity to type-1 IGF receptors. The same receptors are, however, also implicated in the mediation of the growth promoting and proliferative activity of IGF-2, and they may also bind insulin though with significantly less affinity than IGF-1 or IGF-2 (reviewed in Sara et al., 1996; Stewart and Rotwein, 1996).

IGF-1 is a component of the somatotropic axis which mediates many effects of pituitary growth hormone (GH), such as regulation of tissue growth, cell differentiation and metabolic activity. In addition to IGF-1, the somatotropic axis includes hypothalamic growth hormone-releasing hormone (GHRH) and somatostatin, which stimulate and inhibit pituitary GH secretion, respectively. Several members of the somatotropic axis exhibit sleep-modulating activity. Exogenous GHRH enhances sleep, particularly NREMS, in rats (Ehlers et al., 1986; Obál et al., 1988), rabbits (Obál et al., 1988) and humans (Steiger et al., 1992; Kerkhofs et al., 1993), whereas inhibition of endogenous GHRH is followed by suppression of spontaneous sleep (Obál et al., 1991; 1992). Somatostatin stimulates REMS (Danguir, 1986) and decreases NREMS (Beranek et al., 1997). Acute administration of GH stimulates REMS in rats (Drucker-Colín et al.,1975), cats (Stern et al., 1975) and humans (Mendelson et al., 1980). The sleep findings in patients with chronic alterations in GH secretion are variable. Recent observations, however, indicate that chronic overproduction or deficiency in GH is associated with enhancements and decreases in the intensity of NREMS, respectively, as assessed by determining the slow wave activity in the EEG (Åström and Jochumsen, 1989; Åström and Trojaborg, 1992). Immunoneutralization of GH is followed by decreases in sleep in the rat (Obál et al., 1997). Transgenic mice with excess GH production sleep more than normal mice (Lacmanshing and Rollo, 1994). In contrast, mice deficient in the entire somatotropic axis sleep less than their littermates (Zhang et al., 1996). These findings suggest that, particularly in chronic conditions, GH may have NREMS promoting activities, possibly via some metabolic actions, but the responsiveness of NREMS to GH varies with the species and the age of the subjects (reviewed in Obál et al., 1997). Sleep-associated variations in plasma IGF-1 concentrations have been detected in humans (Prinz et al., 1995). The effects of IGF-1 on sleep, however, have not been studied, and therefore it is not known whether IGF-1 has any role in the mediation of the GH-induced sleep alterations.

The aim of our experiments was to study changes in sleep in response to intracerebroventricular (ICV) injection of IGF-1 in rats. To determine if insulin receptors are involved in the mediation of the sleep responses, sleep-wake activity was also recorded after ICV injection of insulin.

METHODS

Animals, surgery

Male Sprague-Dawley rats (300-350 g b.w.) were used. Under ketamine-xylazine anesthesia (87 and 13 mg/kg intraperitoneally, respectively), the rats were implanted with

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stainless steel jewelry screws as electrodes for EEG recording, and a thermistor for recording cortical brain temperature (Tcrt). A guide cannula was implanted into the left lateral ventricle. The position of the cannula was verified during implantation by a sudden drop in resistance against inflow of physiological saline. The placement of the cannula and the drainage of the lateral ventricle was verified by means of the drinking response elicited by ICV injection of angiotensin II (200 ng, 2 µl) a few days before the sleep experiment. At the termination of the sleep recording, trypan blue was injected into the cannula, and the ventricular system was checked for staining. Data were used only from those rats in which the angiotensin tests were positive and the postmortem examination of the brain also confirmed the proper position of the cannula.

Recording

The rats were housed in individual Plexiglas cages in the recording chambers. The ambient temperature was regulated at 26°C, and a 12-12-hour light-dark cycle was maintained with light on at 8:30 a.m. Food and water were continuously available. The rats were allowed 7 to 10 days to recover after surgery. During recovery, the rats were housed in the recording chambers, and they were connected to the recording tethers for habituation. The tethers were attached to commutators. The movements of the rats were recorded by means of electromagnetic transducers attached to the tethers. Cables from the commutators and electromagnetic transducers were connected to amplifiers in an adjacent room. The signals were digitized by an AD converter (64 Hz sampling rate) and stored on a computer. For the evaluation of the states of vigilance, the EEG, motor activity and Tcrt signals were displayed on the computer screen. For each 8-s epoch, the power density spectra were also computed from the EEG. The power density values were determined and displayed in 0.5 Hz bins between 0.5 and 20 Hz. The states of vigilance were scored visually in 8-s intervals (NREMS: high-amplitude EEG slow waves, lack of body movements, and declining Tcrt upon entry; REMS: highly regular theta activity in the EEG, general lack of body movements with occasional twitches, and rapid rise in Tcrt at onset; wakefulness: EEG amplitudes similar but less regular than in REMS, frequent movements, and a gradual increase in Tcrt after arousal). The duration of the states of vigilance was expressed as percent of recording time each hour. Power density values between 0.5 and 4.0 Hz were integrated to characterize EEG slow wave activity (SWA). SWA belonging to artifact-free uninterrupted 8-s epochs of NREMS were averaged and thereby mean SWA during NREMS was computed for each hour. Occasionally, the rats failed to sleep for 1 hour or longer at night. In these particular cases, the mean of the preceding and following hourly SWA was used to fill the missing value for the statistics. The 8-s Tcrt values were averaged for 1 hour intervals.

Experimental protocol

Eight groups of rats were used as follows: Three groups were injected in the light period 6 hours after light onset: 0.05 µg IGF-1 (n=10), 0.5 µg IGF-1 (n=6), and 5.0 IGF-1 (n=10); and five groups were injected at dark onset: 0.05 µg IGF-1 (n=10), 0.5 IGF-1 (n=12), heat-inactivated 0.05 µg IGF-1 (n=7), 0.04 µg insulin (n=8); 0.4 µg insulin (n=8). The doses of insulin (0.04 and 0.4 µg) were selected to match the molar dose of 0.05 and 0.5 µg IGF (0.0065 nmol and 0.065 nmol), respectively. In the rat, sleep propensity is highest at light onset, declines during the day, and is at minimum levels at the beginning of the dark period. Injections at dark and light onsets are generally used to demonstrate increases and decreases in NREMS, respectively, in response to a particular substance (Inoué et al., 1984). It was anticipated that duration of NREMS during the second portion of the light period might be still high enough to detect sleep suppression and already low enough to pick up sleep promotion if IGF-1 exerts these activities. IGF-1 (human recombinant IGF-1) and insulin (human, enzymatically derived from porcin insulin) was obtained from Peninsula Lab, Inc. (CA). For heat inactivation, the IGF-1

Table 1
Duration of NREMS and REMS during 6 Hours after ICV Injection of Physiological Saline (Baseline), IGF-1, Heat-Inactivated IGF-1 (HIGF-1), or Insulin (INS), and the % Change from Baseline in EEG Slow Wave Activity (SWA) during NREMS

				NREMS		REMS		SWA
				Baseline	Experimental	Baseline	Experimental	Difference
				% recording time (6 h)		% recording time (6 h)		%
L	0.05 µg	IGF-1	n=10	48.4 ± 1.87	54.2 ± 1.90*	11.1 ± 1.06	10.6 ± 1.16	+1.2 ± 1.55
L	0.50 µg	IGF-1	n=6	50.8 ± 3.18	50.8 ± 2.30	10.3 ± 0.34	11.6 ± 0.53	+0.1 ± 2.25
L	5.00 µg	IGF-1	n=10	49.4 ± 1.42	47.1 ± 1.52	11.6 ± 0.82	11.5 ± 1.26	+2.3 ± 2.09
D	0.05 µg	IGF-1	n=8	31.5 ± 2.61	37.4 ± 2.51*	3.4 ± 0.86	7.7 ± 1.40*	+2.0 ± 2.70
D	0.50 µg	IGF-1	n=12	33.5 ± 1.72	35.3 ± 1.52	6.2 ± 1.44	5.5 ± 0.85	+3.7 ± 2.29
D	0.05 µg	HIGF-1	n=7	32.4 ± 2.64	28.2 ± 1.04	4.9 ± 0.62	5.4 ± 0.79	+0.2 ± 1.93
D	0.04 µg	INS	n=8	32.7 ± 3.50	27.9 ± 4.72	4.0 ± 0.81	3.0 ± 0.72	+0.3 ± 1.56
D	0.40 µg	INS	n=7	30.7 ± 3.31	35.4 ± 2.23	4.3 ± 1.42	6.6 ± 0.88	-0.3 ± 1.85

* Significantly different from baseline (ANOVA for repeated measures)
Mean ± SE is provided for each value.

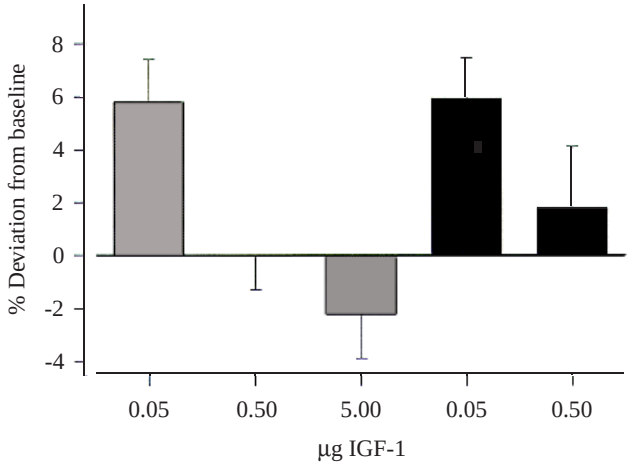

Figure 1. Changes in NREMS (difference from baseline recording; mean ± SE) during the 6 hours after intracerebroventricular injection of various doses of IGF-1 administered 6 hours after the onset of the 12 hour light period (shaded columns) or at the onset of the dark period (closed columns).

solution was exposed to 75°C for 30 min. Both IGF-1 and insulin were dissolved in physiological saline and injected in a volume of 2 µl. Each rat received 2 ICV injections: physiological saline on day 1 (baseline day), and IGF-1 or insulin on day 2 (experimental day). As a growth factor and metabolic hormone, IGF-1 may elicit long-term effects in both glial cells and neurons. IGF-1 or insulin was, therefore, always administered on day 2 of the recording.

ICV injections were performed 10 min before dark onset or 10 min before hour 6 of the light period. The groups injected at dark onset were recorded from for 12 hours in the dark period, and for 11 hours during the subsequent light period. In the groups injected during the light period, recording was started in the morning with light onset, interrupted for injections and then continued during the 6 hours of the second portion of the light period, and for 11 hours at night. If not mentioned otherwise, only the values for a 6 hour postinjection period are reported herein irrespective of the time of the injection; the IGF-1-induced alterations in sleep were over during this period.

Statistics

Two-way analysis of variance (ANOVA) for repeated measures were used to determine the effects of IGF-1 or insulin on a state of vigilance, SWA during NREMS, and Tcrt in a group of rats. Time (6 hour blocks) and treatment (physiological saline or IGF or insulin) were the 2 factors of the ANOVA. Only the results with significant treatment-effects are provided in the current report. Intergroup comparisons (evaluation of the differences in the effects of the various doses of IGF-1) were performed by means of one-way ANOVA followed by the Student-Newman-Keuls test in the light period (3 groups) or by the Student *t*-test in the dark period (2 groups). The Student *t*-test was used to analyze the effects of IGF-1 on the states of vigilance in postinjection hour 1. In all tests, an α level of $p<0.05$ was taken as an indication of statistical significance.

RESULTS

The lowest dose of IGF-1 enhanced NREMS during a 6 hour time block after injection in both the light (ANOVA, $F(1,9)=11.717$, $p<0.05$) and the dark periods ($F(1,9)=13.673$, $p<0.05$) (Table 1, Fig. 1). The increases in NREMS were modest and occurred between hours 1 and 4 postinjection with the most consistent enhancements in hour 2 (dark) or hour 3 (light) postinjection. NREMS was not significantly altered in hour 1 (Student *t*-test). The NREMS-promoting activity of IGF-1 vanished when the dose was raised to 0.5 µg. Although the mean duration of NREMS per 6 hours did not change in response to 5.0 µg IGF-1, this large dose elicited a prompt and significant suppression in NREMS in postinjection hour 1 (% recording time; baseline: 51.7 ± 1.96, IGF-1: 33.0 ± 4.21; $p<0.05$, Student *t*-test). The NREMS loss was recovered in hour 3 postinjection. There were significant differences in the effects on NREMS among the three doses of IGF-1 injected during the light period (one-way ANOVA: $F(2,23)=5.26$, $p<0.05$; Student-Newman-Keuls test: significant difference between the effects of 0.05 µg and 5.0 µg IGF-1) and between the doses administered at dark onset (Student *t*-test).

SWA in NREMS did not change during 6 hours after IGF-1 injection. Interestingly, consistent (+10.4 ± 1.92%) and statistically significant ($F(1,9)=27.424$, $p<0.05$) enhancements in NREMS-associated SWA were found throughout the 11 hour recording during the dark period when 5.0 µg IGF-1 was injected 6 hours after light onset. These changes in SWA were not accompanied by changes in the duration of NREMS.

In general, REMS was not altered by ICV administration of IGF-1 during the day or at night. Increases in REMS were observed after the 0.05 µg IGF-1 injected at dark onset ($F(91,9)=8.457$, $p<0.05$). This group of rats, however, produced less REMS on the baseline day than the rat injected with the larger dose (Table 1).

The low dose of insulin did not alter sleep (Table 1). Although NREMS obviously tended to increase after the administration of 0.4 µg insulin (NREMS enhanced in 6 out of the 7 rats) these changes did not reach the level of statistical significance.

Courses of Tcrt did not differ after physiological saline and IGF-1 or insulin injections.

DISCUSSION

The low dose of ICV IGF-1 elicited modest enhancements of sleep. Increases in NREMS were also reported in response to ICV infusion of insulin (Danguir and Nicolaidis, 1984). The molar dose of IGF-1 was, however, lower than that of insulin required for stimulation of NREMS in our experiments. Therefore, the NREMS-promoting activity of IGF-1 is attributed to stimulation of type-1 IGF-1 receptors and not to insulin receptors. Enhancements in NREMS developed slowly and persisted for several hours after the administration of IGF-1 in both the dark and the light period. This suggests that stimulation of sleep elicited by GHRH is not mediated via GH-IGF-1 for GHRH has a prompt and relatively short-lasting sleep-promoting activity in rats (Ehlers et al., 1986; Obál et al., 1988). In fact, GH-deficiency created by hypophysectomy fails

to interfere with the enhancements in NREMS elicited by GHRH (Obál et al., 1996). It was suggested, therefore, that the NREMS-promoting activities of GHRH and GH are independent: GHRH stimulates NREMS via a neurotransmitter-like action in the basal forebrain, whereas GH alters sleep through some metabolic action. Although the mechanisms might be slightly different, GH, IGF-1, and insulin are all characterized by protein anabolic (Russell-Jones and Umpleby, 1996) and NREMS-promoting activities. NREMS is associated with increased rates of cerebral protein synthesis in the rat (Ramm and Smith, 1990) and monkey (Nakanishi et al., 1997). It cannot be excluded that these phenomena are not only correlative, but causally related. Also, the GH-induced rise in the concentration of IGF-1 in the brain or in the blood (IGF-1 seems to be transported from blood into the brain via the choroid plexus [Reinhardt and Bondy, 1994; Davidson et al., 1990]) may contribute to the sleep alterations found in patients with chronic overproduction of GH. In this respect, the late increases in SWA observed after the high dose of IGF-1 might have particular significance: enhanced NREMS intensity is reported to be a characteristic symptom of patients with high plasma GH concentrations (Åström and Trojaborg, 1992).

Acute administration of GH increases REMS in three species (Drucker-Colín et al., 1975; Stern et al., 1975; Mendelson et al., 1980). The low dose of IGF-1, however, stimulated REMS modestly only in the dark period and failed to do so in the afternoon when REMS is normally high. The time of the day variations in the REMS responses to IGF-1 suggest that IGF-1 has no specific REMS-promoting activity. The increases in REMS at night might result from the combined effects of the low baseline and the increases in NREMS. If this speculation is correct then the mechanism of stimulation of REMS by acute GH injection is independent from IGF-1.

The NREMS-promoting effects of IGF-1 vanished when the dose was increased, and the high dose, in fact, elicited an acute suppression in NREMS in postinjection hour 1. IGF-1 acts as a negative feedback in the somatotropic axis inhibiting GH secretion at levels of both the pituitary and the hypothalamus. In the hypothalamus, IGF-1 stimulates somatostatin (Berelowitz et al., 1981; Becker et al., 1995; Gil Ad et al., 1996; Ghigo et al., 1997). Although an acute release of GHRH is also occasionally found in response to IGF-1 (Aguilla et al., 1993), other papers report that IGF-1 suppresses GHRH secretion (Shibashaki et al., 1986; Becker et al., 1995) and GHRH mRNA levels (Sato and Frohman, 1993; Uchiyama et al., 1994). Inhibition of GHRH might require stronger IGF-1 stimulation than the release of somatostatin (Ghigo et al., 1997). Irrespective of which event occurs first, both an inhibition of GHRH and a stimulation of somatostatin are associated with decreases in NREMS. It seems that it is not the blood-borne IGF-1, but the hypothalamic IGF-1 stimulated by pituitary GH which is involved in the hypothalamic inhibition of the somatotropic axis (Sato and Frohman, 1993). The importance of the intracerebral IGF-1 in the regulation of GH secretion is supported by the observation that ICV administration of IGF-1 in itself (Abe et al., 1983; Tannenbaum et al., 1983) or in combination with IGF-2 (Harel and Tannenbaum, 1992) inhibits GH secretion in the rat. The dose of ICV-injected IGF-1 suppressing GH secretion is 0.5 µg or higher (Becker et al., 1995), i.e., in the dose range of IGF-1 that failed to enhance NREMS or caused a suppression in our experiments. It is suggested therefore, that the attenuation of the sleep-promoting activity of IGF-1, and the appearance of NREMS suppression when the dose of ICV IGF-1 is high is due to a gradually developing inhibition of GHRH and/or stimulation of somatostatin.

In conclusion, in addition to the acute NREMS suppressive effect of IGF-1, our experiments indicate that IGF-1 has a sleep-promoting potential. Experiments with chronic administration of IGF-1 can reveal whether this activity of IGF-1 is involved in the mechanisms of sleep alterations in conditions with permanent overproduction of GH.

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