Time- and Dose-Dependent Responses of Brain Histamine to Intracerebroventricular and Intraperitoneal Administrations of Growth Hormone-Releasing Factor (GRF₁₋₄₄)

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Med., 1987, **151** (4), 429-442 — Changes in the level of histamine (HA) in rat
brain induced by intracerebroventricular (i.c.v.) and intraperitoneal (i.p.) adminis-
trations of growth hormone-releasing factor (GRF₁₋₄₄) were studied. HA was
determined by high-performance liquid chromatography (HPLC) in the anterior
hypothalamic region, posterior hypothalamic region, median eminence, adeno-hypo-
physis, neurohypophysis, hippocampus and prefrontal cortex. GRF₁₋₄₄ (1-10 µg,
i.c.v.) induced significant time- and dose-dependent increases in the concentration
of HA in the hypothalamo-hypophyseal system and time-dependent decrease of
HA in the hippocampus. In contrast, after i.p. administration of GRF₁₋₄₄ (10 µg)
the level of HA in the hypothalamus tended to decrease but the total amount of
H-1 receptors in the hypothalamo-hypophyseal system did not change. Circadian
variations in the GRF-induced HA and growth hormone responses were also
observed, responses being lower in the evening than in the morning. It is conclud-
ed that GRF interacts with HA at the central level to optimize the function of the
somatotropinergic system. — GRF; brain histamine; hypothalamo-
hypophyseal system; GH; H-1 receptors

Histamine (HA) is a biogenic amine that was recently shown to be a neur-
otransmitter (Green et al. 1978; Schwartz et al. 1980), neuromodulator (Cacabelos

1984, 1985a, b; Cacabelos et al. 1985b, 1987) or neuroregulator (Prell and Green 1986). HA cell bodies are located in the posterior region of the hypothalamus (Panula et al. 1984; Steinbusch and Mulder 1984; Watanabe et al. 1984; Senba et al. 1985) sending axons in apparently organized ascending and descending pathways to the cortex (Takeda et al. 1984) and spinal cord (Wahlestedt et al. 1985). Our previous neurochemical studies suggested that there are three main intrahypothalamic HA pathways from the posterior hypothalamus to the anterior hypothalamus, median eminence and neurohypophysis (Cacabelos 1984, 1985b; Cacabelos et al. 1985b), where abundant HA fibers are detected by immunohistochemical methods using antibodies against HA (Steinbusch and Mulder 1984) and histidine decarboxylase (Watanabe et al. 1984; Senba et al. 1985; Inagaki et al., unpublished data). These intrahypothalamic HA pathways are supposed to regulate neuroendocrine functions (Cacabelos 1984, 1985a, b) and particularly corticotropinergic (Cacabelos et al. 1983, 1985b; Cacabelos 1984, 1985b) and vasopressinergic (Cacabelos 1984, 1985b; Cacabelos et al. 1987) systems. However, HA can also influence other neuroendocrine axes, as described in several reviews (Weiner and Ganong 1978; Roberts and Calcutt 1983; Cacabelos 1984, 1985a, b; Donoso and Alvarez 1984; Tuomisto and Mannisto 1985). In their transit to the infundibular region, HA fibers may establish synaptic contacts with cells secreting growth hormone-releasing factor (GRF) located in the vicinity of the nucleus arcuatus (Bloch et al. 1983; Merchenthaler et al. 1984; Meister et al. 1985) influencing the regulation of the somatotropinergic system. In this regard, we have recently reported that GRF₁₋₂₉ can induce time- and dose-dependent changes in the level of HA in the hypothalamo-hypophyseal system (HhS) (Cacabelos et al. 1986a, b, c). At the same time, the i.p. administration of HA can influence the release of growth hormone (GH) (Cacabelos et al. 1986c; Tanaka et al. 1986), enhancing the GRF-induced GH response in rats (Tanaka et al. 1986). Since structure-activity studies revealed that GRF₁₋₂₉ and GRF₁₋₄₄ have different effects in terms of biological potency (Wehrenberg and Ling 1983; Ling et al. 1984), in this work we studied the effects of intracerebroventricular (i.c.v.) and intraperitoneal (i.p.) administrations of GRF₁₋₄₄ on brain HA and H-1 receptors. We also examined circadian variations of the responses of HA and GH to GRF₁₋₄₄.

MATERIALS AND METHODS

Experiment 1

Male Wistar rats (Nihon Dobutsu, Osaka) weighing 150–200 g were used. The animals were housed in an air-conditioned room at $20 \pm 0.5^\circ\text{C}$ and 50–65% humidity with 12-hour lighting from 7 a.m. to 7 p.m. Commercial rat chow and tap water were available ad libitum.

The animals were anesthetized with Nembutal (40 mg/kg, i.p.) and a stainless steel cannulae (G-30) was implanted into their lateral ventricle (Coordinates: 0.6 mm posterior to the bregma; 1.85 mm lateral to the medial suture; and 3.00 mm ventral to the dorsal surface of the skull). After a 7-day recovery period, the animals were given GRF₁₋₄₄ (10 μg in 5 μl 0.9% saline, i.c.v.) between 10 and 11 a.m. and sacrificed by decapitation 5, 15

or 30 min later ($n=5-7$ rats/group). Control animals received the same amount of vehicle ($5\ \mu\text{l}$ 0.9% saline, i.c.v.).

The brains were removed and the anterior hypothalamic region (AHR), posterior hypothalamic region (PHR), median eminence (ME), adenohypophysis (Ah), and neurohypophysis (Nh) of the hypothalamo-hypophyseal system (HhS) were separated under a stereomicroscope (Konan Camera, R & I, K-380). The hippocampus (hip) and prefrontal cortex (PFCx) were also separated. Samples were weighed, homogenized by sonication (7 oc/80 Watts/15 sec; Sonifier B-12, Branson, Danbury, CT, USA) in 10 v/w of ice-cold 3% perchloric acid containing 5 mM EDTA- Na_2 and centrifuged at $10,000 \times g$ for 15 min at 4°C . The supernatants were stored at -80°C for analysis.

HA was measured by high-performance liquid chromatography (HPLC) with a cation exchanger and an automated fluorometric detection system (Yamatodani et al. 1983, 1985b). Samples of $10-50\ \mu\text{l}$ were injected directly into the HPLC column. The minimum detection limit by this method was 0.05 pmol and the inter-assay coefficient of variation was about 5%.

Experiment 2

Similar rats to those for *Experiment 1* were treated i.c.v. with 1, 5 or $10\ \mu\text{g}$ of GRF₁₋₄₄ ($n=5-7$ rats/group) and the concentrations of HA in the same brain regions as for *Experiment 1* were determined.

Experiment 3

Rats received an i.p. injection of GRF₁₋₄₄ ($10\ \mu\text{g}$) and HA in the HhS was determined 5, 15 and 30 min later as described above.

Experiment 4

Rats were i.p. given 1, 5 or $10\ \mu\text{g}$ of GRF₁₋₄₄ ($n=5-7$ rats/group), and the dose-dependence of changes in HA in the HhS at 15 min was examined.

Experiment 5

The animals were treated daily with GRF₁₋₄₄ ($10\ \mu\text{g}$, i.p.) for 7 days and histamine H-1 receptors in the HhS were measured 30 min after the last treatment. HA H1-receptor binding was assayed by a modification of the method of Chang et al. (1978) and Tran et al. (1978) as described previously (Cacabelos 1985b; Cacabelos et al. 1985b).

Experiment 6

One group of rats was treated with $10\ \mu\text{g}$ of GRF₁₋₄₄ (i.c.v. or i.p.) at 10:00 a.m. and a second group of rats was treated with the same amount of GRF₁₋₄₄ at 8:00 p.m. The rats were sacrificed 0, 5, 10, 15, and 30 min later ($n=5-6$ rats/subgroup). Blood was collected from the trunk in chilled, heparinized plastic tubes and treated with $50\ \mu\text{l/ml}$ of *o*-phenanthroline solution as described elsewhere (Cacabelos 1985b; Cacabelos et al. 1985a). GH plasma levels were determined by radioimmunoassay using kits for rat GH from the National Institute of Arthritis, Metabolism and Digestive Diseases. The sensitivity of the assay was 0.3-0.7 ng GH/tube and the intra-assay and inter-assay coefficients of variation were 9.45% and 12.5%, respectively. The posterior and anterior hypothalamic regions were isolated and their HA levels were measured as in *Experiments 1, 2, 3, and 4*.

Statistics

Experimental data were analyzed statistically by the Student's *t*-test, analysis of variance (ANOVA) and the Mann-Whitney test using an NEC PC-9801VM2 computer.

RESULTS

Effects of i.c.v. GRF₁₋₄₄ on the concentration of histamine in the hypothalamo-hypophyseal system, hippocampus and prefrontal cortex

GRF₁₋₄₄ induced time-dependent changes in the concentration of HA in the AHR and PHR with maxima at 15 min (Fig. 1). In the ME (Fig. 1), Ah and Nh (Fig. 2) maxima were also observed at 15 min. In the hip, GRF₁₋₄₄ produced a significant decrease in the level of HA with a minimum at 15 min (1.35 ± 0.23 vs. 0.42 ± 0.01 nmole/g; $t=9.03$, $p<0.01$), while in the PFCx, HA increased at 5 min

Fig. 1. GRF-induced histamine response in the anterior hypothalamic region, posterior hypothalamic region and median eminence.

GRF₁₋₄₄ (10 μ g) was administered i.c.v. and rats were sacrificed 5, 15 and 30 min later ($n=5-7$ rats/group). For measurement of the dose-dependence of the response of HA animals were sacrificed 15 min after GRF₁₋₄₄ injection. ** $p<0.01$ vs. control (0).

Fig. 2. GRF-induced histamine response in the adenohypophysis and neurohypophysis.

Animals were treated as for Fig. 1 (see also Materials and Methods). $n=5-7$ rats/group; ** $p<0.01$ vs. control (0).

(0.51 ± 0.01 nmole/g; $t=28.46$; $p<0.01$) and decreased later to below the control value (0.33 ± 0.05 nmole/g). These changes were dose-dependent. GRF₁₋₄₄ elicited maximum responses at a dose of $10 \mu\text{g}$ in the HhS (Figs. 1 and 2). The ED₅₀ values were approximately 5×10^{-5} M in the hypothalamus and Ah, 2×10^{-5} M in the ME, and 4×10^{-5} M in the Nh.

Effects of i.p. GRF₁₋₄₄ on the concentration of histamine in the hypothalamo-hypophyseal system

The i.p. administration of GRF₁₋₄₄ induced significant decreases in the concentration of HA in the AHR and PHR at 30 min (Table 1). In the ME, HA increased from 5.60 ± 0.10 (control) to 10.34 ± 2.25 nmole/g ($t=4.706$; $p<0.01$) and 14.75 ± 3.15 nmole/g ($t=6.80$; $p<0.01$) after 15 and 30 min, respectively (Table 1). In the Ah, the HA levels were decreased after 5 min and increased after 15 min (Table 1). In the Nh, the HA level was increased after 5 and 15 min. In the hypothalamus the GRF-induced HA response was dose-dependent with

TABLE 1. *Effects of i.p. administration of GRF₁₋₄₄ on histamine contents in the hypothalamo-hypophyseal system*

Region	Control	5 min	15 min	30 min
Anterior hypothalamic region	1.59 ± 0.15	1.35 ± 0.13	1.41 ± 0.23	0.98 ± 0.05**
Posterior hypothalamic region	2.35 ± 0.35	2.20 ± 0.16	2.07 ± 0.19	1.24 ± 0.03**
Median eminence	5.60 ± 0.10	5.65 ± 0.03	10.34 ± 2.25**	14.75 ± 3.15**
Adenohypophysis	0.70 ± 0.008	0.59 ± 0.09	1.02 ± 0.14**	0.73 ± 0.08
Neurohypophysis	3.86 ± 0.56	5.99 ± 0.42**	8.23 ± 1.76**	4.03 ± 0.59

Dose : 10 µg GRF₁₋₄₄ (i.p.).

Histamine units : nmole/g (mean ± s.d.).

n = 5-7 rats/group.

**p < 0.01 vs. control.

TABLE 2. *Dose-dependent response of brain histamine to i.p. administration of GRF₁₋₄₄*

Region	Dose (µg GRF ₁₋₄₄ , i.p.)			
	0	1	5	10
Anterior hypothalamic region	1.59 ± 0.15	1.13 ± 0.14**	1.15 ± 0.005**	1.41 ± 0.23
Posterior hypothalamic region	2.35 ± 0.35	1.61 ± 0.15**	1.68 ± 0.08**	2.07 ± 0.19
Median eminence	5.60 ± 0.10	5.15 ± 0.96	6.61 ± 1.17	10.34 ± 2.25**
Adenohypophysis	0.70 ± 0.008	0.55 ± 0.04**	0.69 ± 0.09	1.02 ± 0.14**
Neurohypophysis	3.86 ± 0.56	3.98 ± 0.91	5.18 ± 0.88**	8.23 ± 1.76**

Time : 15 min.

Histamine units : nmole/g (mean ± s.d.)

n = 5-7 rats group.

**p < 0.01 vs. control (0).

maximum inhibition at low doses of GRF₁₋₄₄ (Table 2). Increasing doses of GRF₁₋₄₄ potentiated the response of HA in the ME, Ah, and Nh (Table 2) with ED₅₀ values of 9.9×10^{-6} M, 1.3×10^{-5} M, and 1.1×10^{-5} M, respectively.

Effects of i.p. GRF₁₋₄₄ on [³H]-mepyramine binding in the hypothalamo-hypophyseal system

The administration of GRF₁₋₄₄ at a daily dose of 10 µg (i.p.) for 7 days did not modify the total amount of HA H-1 receptors in any region of the HhS (Table 3).

Circadian oscillations in the GRF-induced HA and GH responses

When administered i.c.v. in the morning, GRF₁₋₄₄ induced significant

TABLE 3. *Effect of i.p. GRF₁₋₄₄ on [³H]-mepyramine binding in the hypothalamo-hypophyseal system*

Region	Control	GRF ₁₋₄₄
Anterior hypothalamic region	13.25 ± 0.85	15.25 ± 3.35
Posterior hypothalamic region	14.95 ± 2.55	16.10 ± 3.20
Median eminence	45.85 ± 7.15	49.15 ± 4.85
Adenohypophysis	18.40 ± 6.80	10.50 ± 4.05
Neurohypophysis	57.15 ± 9.15	66.10 ± 5.90

Dose: 10 µg GRF₁₋₄₄/day for 7 days, (i.p.).

Fig. 3. Circadian variations in the GRF-induced histamine response in the anterior and posterior hypothalamic regions. The animals received an i.c.v. injection of GRF₁₋₄₄ at 10:00 a.m. (□) or 8:00 p.m. (▨), as described in Materials and Methods, and the GRF-induced histamine responses in the anterior and posterior hypothalamic regions were measured 5, 15, and 30 min later.

$n=5-7$ rats/group. †† $p<0.01$ vs. control (0). * $p<0.05$ and ** $p<0.01$ vs. corresponding value at 8 p.m.

Fig. 4. Circadian variations in the GRF-induced GH response in plasma.

The animals were treated with GRF₁₋₄₄ (10 µg, i.p. (●) or i.c.v. (▲)) at 10:00 a.m. or 8:00 p.m. (i.p. = (○); i.c.v. = (△)) and plasma GH level was measured by RIA 5, 10, 15 and 30 min later ($n=5-6$ rats/group). For examination of the dose-dependence of GH secretion (stippled bars) animals ($n=6$ rats/group) were killed 7 min after GRF i.p. injection.

increases in the concentration of HA in the AHR after 15 min (Fig. 3), but when given in the evening no response was observed (Fig. 3). The circadian variation in the GRF-induced HA response was most significant after 15 min (Fig. 3), but in the PHR, clear circadian changes were observed after 5, 15 and 30 min (Fig. 3). In this region, the basal and GRF-stimulated HA levels were both lower in the evening than in the morning (Fig. 3).

Circadian variations in the GRF-induced GH response were also highly significant. In the morning i.p. GRF elicited a high peak of GH at 5–15 min (Fig. 4) while in the evening the GRF-induced GH response was less significant. The i.c.v. injection of GRF did not elicit a response in the morning and tended to decrease the plasma GH levels in the evening (Fig. 4). The response of GH to GRF (i.p.) was dose-dependent in the morning ($ED_{50}=4.5 \times 10^{-7}$ M; $E_{max}=2.0 \times 10^{-9}$ M) (Fig. 4) but not in the evening.

DISCUSSION

According to our data, GRF₁₋₄₄ can induce changes in the concentration of

HA in the CNS (Figs. 1, 2, 3) when administered i.c.v. The GRF-induced HA response was found to be time- and dose-dependent in five different regions of the HhS (Figs. 1, 2) but the concentration of GRF necessary to increase the levels of HA ($2-5 \times 10^{-5}$ M) was higher than that necessary to induce the release of GH in vivo ($ED_{50} = 1.9 \times 10^{-7}$ M) or in vitro ($ED_{50} = 1.2 \times 10^{-11}$ M) in rats (Wehrenberg and Ling 1983; Guillemin et al. 1984; Ling et al. 1984) and in monkeys (Almeida et al. 1984). The HA response to GRF in the ME coincided with the GRF-induced GH response in the plasma. In the hypothalamus, the HA response was 5-10 min later than the release of GH from the Ah.

In in vitro studies (Guillemin et al. 1984a, b; Ling et al. 1984) GRF₁₋₄₄ was the most potent of the C-terminal deleted analogs tested in a normal pituitary cell monolayer culture system ($ED_{50} = 12$ pM; $E_{max} = 100$ pM) (Guillemin et al. 1984a, b). In our studies, GRF₁₋₂₉ and GRF₁₋₄₄ were approximately equipotent (GRF₁₋₂₉ $E_{max} = 1.5 \times 10^{-4}$ M; GRF₁₋₄₄ $E_{max} = 2 \times 10^{-4}$ M) in eliciting changes of HA in the brain (Cacabelos et al. 1986a, b, c; Tanaka et al. 1986). Although the GRF receptors mediating GH secretion at the pituitary level and those mediating central actions may differ, a similar amino-terminal portion of the GRF molecule is most probably involved in the binding to the receptors, and deletion of the C-terminal residues 30-44 of GRF does not seem to affect its biological potency in releasing brain HA in the rat.

The i.p. injection of GRF₁₋₄₄ resulted in decreases in the HA levels in the hypothalamus (Table 1). The HA responses in the ME and Nh were higher after i.p. injection of GRF than after its i.c.v. injection. This suggests that although GRF partially crosses the blood-brain barrier (bbb) (Banks and Kastin 1985), its main direct action after its i.p. administration is on areas of the HhS located outside the bbb (i.e., the ME and hypophysis) (Meisenberg and Simmons 1983). In these regions, it may activate histaminergic and somatotropinergic short feedback loops (Cacabelos et al. 1986b) that influence the release of HA at the hypothalamic level. The intrinsic mechanism of interaction of GRF with neuronal HA is still unknown. However, what seems clear is that when administered by different routes (i.e., i.p., i.c.v.) GRF has opposite effects on the HA response in the hypothalamus but similar effects on the response of HA in the ME and hypophysis. This fact provides indirect evidence that GRF may have different physiological roles in the central and peripheral compartments with regard to its interaction with the histaminergic system.

The effect of HA on GH release is still controversial. In a recent review, Tuomisto and Mannisto (1985) suggested that HA is not an important neurotransmitter in the regulation of GH secretion in humans (Carlson and Chang 1980). However, when pituitary glands are incubated with hypothalamic fragments, HA at concentrations of 10^{-8} - 10^{-7} M stimulates the release of GH (Hall et al. 1984) and this effect can be blocked by the H-1 antagonist diphenhydramine (Hall et al. 1984). We have reported that i.p. HA at a dose of 6 μ mole/kg induces a

significant increase in the basal level of GH in rats, and that this effect is abolished by famotidine, a specific H-2 antagonist (Cacabelos et al. 1986c; Tanaka et al. 1986). Our data suggest that at least at the pituitary level, HA can influence the release of GH in vivo through H-2 receptors (Cacabelos et al. 1986c; Tanaka et al. 1986). This is partially in agreement with the fact that although GRF stimulates the release of HA in the HhS, the repeated administration of the neuropeptide does not alter the total amount of H-1 receptors (Table 3). Desensitization to GRF associated with down-regulation of GRF-binding sites (Bilezikjian et al. 1986) cannot be ruled out, because chronic exposure of the pituitary to maximum concentrations of GRF causes partial loss of responsiveness in vivo (Gelato et al. 1985; Wehrenberg et al. 1986) and in vitro (Bilezikjian and Vale 1984). However, in our studies neither single nor repeated doses of GRF affected [^3H]-mepyramine binding in different regions of the HhS where i.p. administration of GRF induced HA responses.

Netti and coworkers (1982, 1984) have reported that HA can inhibit pulsatile GH secretion through a central mechanism, although i.c.v. administration of HA, H-1 and H-2 agonists or antagonists had no effect on the basal plasma GH level (Netti et al. 1981; Knigge et al. 1984). These workers suggested that HA has an inhibitory influence on GRF (Netti et al. 1984), a possibility that is not consistent with the report by Tanaka et al. (1986) that HA potentiates the GRF-induced GH response at 10 min. In connection with these discrepant findings, it should be mentioned that HA probably plays some role in generating an ultradian rhythm of GH secretion through an interaction with GRF and somatostatin. In the rat, GH secretion shows an endogenous ultradian rhythm with secretory bursts occurring at 3-h intervals (Tannenbaum and Ling 1984). In contrast, hypothalamic HA has a circadian rhythm with a minimum in the evening and maximum in the morning in rats (Wada and Cacabelos 1984; Wada et al. 1985; Yamatodani et al. 1985a). In our studies, both HA and GH responded to GRF stimulation in the morning, when the basal HA levels in the hypothalamus are relatively high, but did not respond to GRF in the evening, when the levels of HA in the posterior hypothalamus are lowest (Wada et al. 1985; Yamatodani et al. 1985a). Studies are required on whether HA is responsible for the failure of GH to respond to GRF stimulation in the evening. The influence of somatostatin in these circadian variations of GH and HA in response to GRF must be taken into account, because GRF itself can stimulate the release of somatostatin in the ME and this stimulation of somatostatin release may be why GRF exerts a negative ultrashort-loop feedback to inhibit GH release (Tannenbaum 1984; Tannenbaum and Ling 1984; Aguila and McCann 1985). But actually we do not know whether this effect influences the HA circadian rhythm.

Several conclusions can be made from the present results:

- (1) GRF₁₋₄₄ can induce dose- and time-dependent changes in the concentration of HA in the HhS. Since changes in the concentration of HA in the ME and

hypophysis coincide with the GH response to GRF and since the HA response in the hypothalamus occurs 5–10 min later, the central stimulation of HA may have an inhibitory effect on the secretion of GH after the secretory burst induced by GRF.

(2) The GRF-induced HA response in the hypothalamus differs depending on whether GRF is administered i.c.v. or i.p., but the HA responses in the ME and hypophysis are the same irrespective of the route of GRF administration. These observations indicate that GRF plays different interactive roles with HA in the central and peripheral compartments.

(3) GRF does not affect [³H]-mepyramine binding in the HhS. Thus the effects of HA in response to GRF may not be mainly mediated through H-1 receptors.

(4) Both GH and HA have a blunted response to GRF in the evening. This finding suggests some functional interactions between HA and GRF-somatostatin to elicit an ultradian rhythm of GH secretion which in rats is more apparent in the morning than in the evening.

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