Role of the neuronal histaminergic system in the regulation of somatotropic function: comparison between the neonatal and the adult rat

R Grilli, V Sibilia, A Torsello, F Pagani, M Guidi, M Luoni, C Netti and E E Müller

Department of Pharmacology, School of Medicine, University of Milan, 20129 Milan, Italy

(Requests for offprints should be addressed to E E Müller, Department of Pharmacology, School of Medicine, University of Milan, via Vanvitelli 32, 20129 Milan, Italy)

Abstract

To study possible age-related differences in the role of neuronal histaminergic pathways in the control of GH secretion, the effects of α-fluoromethylhistidine (α-FMH), an irreversible inhibitor of histamine (HA) synthesis, were examined on basal and opioid-induced GH release in neonatal and adult rats. The mechanisms involved in such effects were evaluated by measuring pituitary GH mRNA levels and hypothalamic levels of GH-releasing hormone (GHRH) and somatostatin (SRIF) mRNAs. Daily injection of α-FMH (20 mg/kg, s.c.) in pups of either sex, from birth until 10 days of age, caused a significant increase in baseline plasma GH and potentiated the GH response to the [Met⁵]-enkephalin analog FK 33–824 (1 mg/kg, s.c.) administered 3 h after the last α-FMH injection. GH and SRIF mRNA levels were significantly higher in α-FMH-treated pups than in controls, whereas no difference was observed in GHRH mRNA levels. In young adult male rats, acute administration of α-FMH (100 mg/kg, s.c., 3 h before) did not change significantly basal GH levels but potentiated FK 33–824 (0.3 mg/kg, intracarotid)-induced stimulation of GH secretion. Repeated administration of α-FMH (200 μg/rat, i.c.v., for 3 days) failed to modify basal and FK 33–824-induced GH secretion, caused a significant reduction in hypothalamic GHRH mRNA levels and left SRIF and GH mRNAs unchanged.

These findings indicate that HA exerts an inhibitory effect on GH secretion in both neonatal and adult rats. The different effects of short-term HA depletion on hypothalamic and pituitary indices of somatotropic function observed at the two age periods may be ascribed to the immaturity of the HA system in early postnatal life and to a different functional role of GH-regulatory factors during ontogeny.

Journal of Endocrinology (1996) 151, 195 201

Introduction

It has been reported that growth hormone (GH) concentrations in blood of neonatal rats are high and relatively constant (Cocchi et al. 1976) whereas in adult rats GH secretion is pulsatile (Tannenbaum & Martin 1976). These age-related differences in GH secretory pattern have been ascribed to ontogenic changes in the hypothalamic control of GH secretion. A tonic hypothalamic stimulation presumably mediated by GH-releasing hormone (GHRH) prevails in the perinatal period (Khorram et al. 1983) while later in life a complex interaction between GHRH and somatostatin (SRIF) gives rise to the rhythmic GH secretion (Tannenbaum & Ling 1984, Plotksy & Vale 1985). Characterization of the central neurotransmitter and peptidergic pathways involved in the regulation of GH secretion throughout life is still a matter of interest. Among the various neurotransmitters participating in the control of GH, the central histaminergic system has been previously reported to exert an inhibitory action on the physiological and pharmacologically induced GH secretion in adult rats (Netti et al. 1981, 1982). However, the mechanisms underlying the GH inhibitory effects of histamine (HA) and the possible significance of neuronal histaminergic pathways in GH control during the immediate postnatal period have yet to be established. The developmental profile of HA associated with nerve terminal function resembles that of other monaminergic transmitters, starting from low level at birth and increasing progressively to adult values within the first 2–3 weeks of postnatal life (Subramanian et al. 1981). An effective depletion of the neurotransmitter pool of HA in the immature nervous system can be obtained by daily administration of the specific irreversible inhibitor of the HA-synthesizing enzyme, α-fluoromethylhistidin (α-FMH), which has been reported to significantly decrease hypothalamic HA by postnatal day 9 (Slotkin et al. 1983). Therefore, in the present study, we examined whether or not impairment of
histaminergic function by α-FMH in the developing rat brain could influence GH secretion and some of the hypothalamic–pituitary indices of somatotropic function. The effect of α-FMH was evaluated on basal and stimulated GH secretion, the latter accomplished via the [Met⁶]enkephalin analog FK 33–824 since opioids are able to elicit GH secretion in neonatal as well as adult rats (Kuhn & Schanberg 1981, Kuhn & Bartolome 1983, Arce et al. 1991). To verify potential age-related differences in the mechanisms responsible for the modulatory action of HA on GH secretion, a similar study was performed in adult rats.

Materials and Methods

Animals

Male and female 10–day-old and young-adult male (3 months old) Sprague–Dawley rats (Charles River Breeding Laboratories, Calco, Italy) were used. Pups were received on the day of birth (day 1) and randomly divided among dams (eight to nine/dam). All rats were housed under controlled conditions of temperature (22 ± 2 °C), humidity (65%) and light (lights on from 0600 to 2000 h). All adult rats had free access to a standard dry diet and tap water.

All experiments were performed in accordance with the Italian Guidelines for the Use of Animals in Medical Research.

Drugs

The compounds used were: α-FMH, a gift from Merck Sharp & Dohme, Rome, Italy, and FK 33–824 ([In-Ala², Me-Phe⁶]-Met-(5)⁵-oil-enkephalin), a gift from Sandoz, Basel, Switzerland. All drugs were dissolved in saline and injected in the following volumes: 5 µl/rat i.c.v. for α-FMH; 1 ml/kg i.p. for α-FMH; 2 ml/kg s.c. for FK 33–824; 1 ml/kg intracarotid (i.a.) for FK 33–824.

Experimental procedure

Infant rats

Pups were randomly divided into two groups (32 rats/group) and treated daily with saline or α-FMH (20 mg/kg, s.c.), a dose reported to be effective in reducing HA levels in the hypothalamus (Garberg et al. 1980). All treatments were initiated on day 1 and continued for 9 days.

Pups were weighed daily and on day 10 the tail length was also measured. Body weight and tail length were taken as indices of body growth.

On day 10, 2 h after the last injection of α-FMH and 1 h before the experiment, all pups were separated from their dams. Sixteen of the α-FMH–treated and 16 saline–treated pups were injected with FK 33–824 (1 mg/kg, s.c.) and the remaining received isovolumetric amounts of saline. After 15 min, all pups were killed by decapitation and trunk blood was collected. The pituitary gland and the whole hypothalamus (a region bordered dorsally by the thalamus, rostrally by the optic chiasm and caudally by the mammary bodies) were quickly dissected out, frozen on dry ice and stored at −80 °C until processed for mRNA extraction.

Adult rats

Under ether anesthesia, carotid catheters were placed into all the rats for blood sampling 3 days before the experiments. Rats given drugs i.c.v. had polyethylene cannulae implanted into the left lateral ventricle of the brain 1 week before the experiment as previously described (Netti et al. 1981). Animals were tested between 1130 and 1300 h to minimize the interference by spontaneous ultradian fluctuations in response to the stimulus. Under our experimental conditions, GH levels are low at this time (Netti et al. 1982).

In a first series of experiments, the effects of a single administration of α-FMH (100 mg/kg, i.p.) on basal and FK 33–824–induced GH release were studied. This dose of α-FMH was previously shown to be effective in altering GH secretion (Sibilla et al. 1993). α-FMH was injected 3 h before FK 33–824 (0·3 mg/kg, i.a.). In a second series of experiments to study the specific effects of central HA impairment on the somatotropic function, α-FMH was administered once daily for 3 days at a dose (200 µg/5 µl per rat) previously shown to be effective in reducing brain HA levels (Garberg et al. 1980, Duggan et al. 1984) and to affect pituitary hormone secretion (Kamei et al. 1993, Sibilla et al. 1993, Knigge et al. 1994). Control rats were given isovolumetric amounts of sterile physiological saline.

At 3 h after the last treatment with α-FMH or saline, rats were given FK 33–824 (0·3 mg/kg, i.a.). Blood samples (0·25 ml) were collected at 0, 10, 15, 20 and 40 min after FK 33–824 or saline, the blood being replaced each time with an equal volume of heparinized saline. Plasma was separated and stored at −20 °C until assayed.

At the end of the experiment all rats were killed by decapitation and the anterior pituitary and the hypothalamus were quickly dissected out as previously described, frozen on dry ice and stored at −80 °C until processed for RNA extraction.

GH assay

Plasma GH concentrations were measured by RIA as previously described (Schalch & Reichlin 1966) with materials kindly provided by the NIDDK of the National Institutes of Health, Bethesda, MD, USA.

Values are expressed in terms of NIDDK-rat-GH-RP-2 standard (potency 2 IU/mg) as µg/l of plasma. The minimum detectable value of rat GH was 1·0 µg/l; intra-assay variability was 6%. To avoid interassay variations, samples from each experiment were assayed within one RIA.
**GH, GHRH and SRIF mRNA levels**

Total RNA was isolated from each pituitary or hypothalamus by the single-step acid guanidinium thiocyanate–phenol–chloroform extraction method (Chomczynski & Sacchi 1987). Total RNA samples (15 μg/sample) from the pituitary were run on a 1:2% formaldehyde/agarose gel and transferred to nylon membranes (Hybond N, Amersham, Little Chalfont, Bucks, UK).

The membranes were hybridized with a cDNA probe specific for rat GH (kindly provided by F De Noto, University of California, San Francisco, CA, USA). Total RNA samples (15 μg/sample) from the hypothalamus were loaded on a slot blotter and the nylon membranes were hybridized with cDNA probes specific for rat GHRH (kindly provided by Dr K E Mayo, Northwestern University, Evanston, IL, USA) and rat SRIF (kindly provided by Dr R H Goodman, Oregon Health Center, Portland, OR, USA). The specificity of the GHRH and SRIF probes was assessed in preliminary Northern blots in which both GHRH and SRIF mRNAs appeared as single transcripts of the expected molecular size. Probes were labeled by random primer (Rediprime, Amersham) with [α-32P]dCTP to a specific activity of 1 × 10^9 d.p.m./μg DNA.

To control for homogeneity of RNA loading, the nylon filters were then stripped and hybridized with [α-32P]dCTP-labeled rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe.

Autoradiography was carried out at −70 °C with intensifying screens for 1–6 h for GH and for 3–6 days for GHRH and SRIF.

Quantification of hybridization signal was performed using a computerized image analyzer, an acquisition system driven by a Macintosh computer and the 'Image' software (written by Wayne Rasband and kindly provided by the National Institutes of Health).

GH, GHRH and SRIF mRNA levels were normalized for RNA loading and expressed as a percentage of the respective control group values.

**Statistical analysis**

Values for each experimental group were pooled and expressed as mean ± S.E.M. The area under the GH response-time curve (AUC) was calculated by trapezoidal approximation. Differences between groups were assessed by one-way ANOVA followed by Student’s t-test or Tukey test, with P<0.05 considered statistically significant.

**Results**

**Infant rats**

Daily administration of α-FMH did not influence the growth of the pups, since at postnatal day 10 no differences in body weight (Fig. 1) or tail length (data not shown) were recorded in α-FMH-treated vs control pups. Treatment with α-FMH significantly enhanced basal plasma GH levels (171 ± 64–6% increase over control; P<0.05) measured on day 10, 3 h after the last injection of the drug (Fig. 2).

In both pup groups, challenge with FK 33–824 induced a significant (P<0.05) increase in GH secretion but in the α-FMH-pretreated pups the GH-releasing effect of FK 33–824 was strikingly enhanced (plasma GH: 54.2 ± 9.9 and 121.6 ± 28 μg/l for FK 33–824 and α-FMH+FK 33–824 respectively; P<0.05) (Fig. 2).

As shown in Table 1, GH mRNA levels were significantly higher in the pituitary of α-FMH-treated pups than in that of controls (23.8 ± 8.7% increase over control; P<0.05). Similar results were obtained for hypothalamic SRIF mRNA levels (18.7 ± 9.0% increase over control; P<0.05; Fig. 3 and Table 1), whereas no significant differences were observed in GHRH mRNA levels (Table 1).

**Adult rats**

In young-adult male rats, since systemic administration of α-FMH had no effect on basal plasma GH levels, basal values measured in saline- and α-FMH-treated rats were
increased GH secretion was evident when considering both the peak plasma GH concentrations (peak plasma GH: 67.1 ± 18.4 and 154.0 ± 23.4 µg/l for FK 33–824 and α-FMH+FK 33–824 respectively; P<0.05) (Fig. 4) and the integrated GH secretion (AUC: 1811 ± 171 and 3326 ± 691 µg/l per 40 min for FK 33–824 and α-FMH+FK 33–824 respectively, P<0.05) (Fig. 4, inset).

In contrast, a 3-day i.c.v. treatment with α-FMH did not modify either basal plasma GH levels or the effect of the FK 33–824 challenge (Fig. 5).

No differences in GH and SRIF mRNA levels were observed in adult rats treated i.c.v. with α-FMH or vehicle, whereas a significant reduction (P<0.05) in GHRH mRNA levels was present in α-FMH-treated rats (26.7 ± 5.3% decrease vs control; P<0.05) (Table 2).

Acute or short-term administration of α-FMH and acute administration of FK 33–824 did not induce untoward behavioral effects in infant or young-adult rats.

**Discussion**

The results of the present study demonstrate for the first time the involvement of the histaminergic system in the control of GH secretion in the neonatal rat. Functional impairment of the histaminergic system from birth by α-FMH-induced inhibition of HA synthesis caused an
increase in basal and opioid-induced GH secretion. Probably, the increase in plasma GH levels in HA-depleted neonatal rats was the result of enhanced GH synthesis since GH mRNA levels in the pituitary of α-FMH-treated rats were significantly higher than in controls.

The mechanism underlying the above results is presumably unrelated to a direct action of the histaminergic system on somatotrophs, since HA is unable to influence GH secretion from the pituitary in vitro (Hall et al. 1984) and exerts an inhibitory effect on GH secretion in adult rats only when injected i.c.v. (Netti et al. 1981, 1982). Therefore, the increase in the releasable GH pool after removal of the inhibitory histaminergic tone is probably due to enhanced activity of hypothalamic GHRH neurons which in neonatal rats regulate not only the release but also the synthesis of GH (Cozzi et al. 1986).

Table 2 Effect of α-FMH treatment on GHRH, SRIF and GH mRNA levels in the pituitary of young-adult rats. Three-month-old male rats were given α-FMH (200 µg/rat per day, i.c.v.) or isovolumetric amounts of physiological saline for 3 days. Data are expressed as percentages of the respective control value. The number of replicates is shown in parentheses. The GHRH/GAPDH, SRIF/GAPDH and GH/GAPDH signal ratios (mean ± s.e.m.) in the control lanes were 0.434 ± 0.03, 0.88 ± 0.16 and 1.36 ± 0.16 respectively.

<table>
<thead>
<tr>
<th>Levels (%)</th>
<th>Control</th>
<th>α-FMH</th>
</tr>
</thead>
<tbody>
<tr>
<td>GHRH mRNA</td>
<td>94.4±14.8 (9)</td>
<td>73.2±5.3 (12)*</td>
</tr>
<tr>
<td>SRIF mRNA</td>
<td>94.4±14.8 (9)</td>
<td>84.7±8.7 (12)</td>
</tr>
<tr>
<td>GH mRNA</td>
<td>94.4±14.8 (9)</td>
<td>73.2±5.3 (12)*</td>
</tr>
</tbody>
</table>

*P<0.05 vs control.

Figure 4 Effect of acute α-FMH treatment on basal and stimulated plasma GH concentrations in young-adult male rats. Three-month-old male rats were given α-FMH (100 mg/kg, i.p.) or isovolumetric amounts of physiological saline 3 h before being challenged with FK 33–824 (0.3 mg/kg, i.a.) or saline. Main panel: plasma GH concentrations (µg/l) are shown as the mean ± s.e.m. of five determinations. ○, α-FMH; △, FK 33–824; ▲, α-FMH+FK 33–824. Inset: integrated GH secretion in the 40 min sampling period. Data are expressed as the mean ± s.e.m. of five determinations. Open bar, α-FMH; stippled bar, FK 33–824; solid bar, α-FMH+FK 33–824. *P<0.05 vs control; **P<0.05 vs FK 33–824 alone.

Figure 5 Effect of 3-day α-FMH treatment on basal and stimulated plasma GH concentrations in young-adult male rats. Three-month-old male rats were given α-FMH (200 µg/rat per day, i.c.v.) or isovolumetric amounts of physiological saline for 3 days. On the fourth day, rats were challenged with FK 33–824 (0.3 mg/kg, i.a.) or saline. Main panel: plasma GH concentrations (µg/l) are shown as the mean ± s.e.m. of five determinations. ○, α-FMH; △, FK 33–824; ▲, α-FMH+FK 33–824. Inset: integrated GH secretion in the 40 min sampling period. Data are expressed as the mean ± s.e.m. of five determinations. Open bar, α-FMH; stippled bar, FK 33–824; solid bar, α-FMH+FK 33–824. *P<0.05 vs control.
The unchanged hypothalamic GHRH mRNA levels after α-FMH injection are not incompatible with this view; in fact, HA could have influenced exclusively the release and not the synthesis of GHRH. Moreover, a regulatory action of HA on other hypothalamic messengers such as γ-aminobutyric acid and thyrotropin–releasing hormone may also be responsible for the elevated plasma GH levels in neonatal rats (Ács et al. 1990). In this context, it is of interest to recall colocalization of histidine decarboxylase and glutamate decarboxylase in numerous perikarya of the magnocellular nucleus of the posterior hypothalamus (Takeda et al. 1984) and the reported increase in thyrotropin secretion in rats treated with α-FMH (Tuominen et al. 1996).

Alternatively, a stimulatory action on GHRH mRNA induced by HA deprivation could be masked by the counteracting feedback effect on GHRH function of enhanced circulating levels of GH (Müller 1987). Consistent with the hypothesis of a self-regulating GH system is the seemingly paradoxical increase in hypothalamic SRIF mRNA levels. Since endogenous SRIF does not play a functional role in regulating GH release in the immediate postnatal period of the rat (Rieutort 1981), it is not surprising that GH secretion remained elevated in spite of the increase in hypothalamic SRIF synthesis and, probably, release.

The apparently contradictory results obtained in neonatal versus adult rats might be explained by the ontogenic development of the hypothalamic GH regulatory factors. The lack of an increase in basal GH levels after acute blockade of HA synthesis by α-FMH in adult rats could be due to the timing and duration of blood sampling. These experiments were performed at a time when GH secretion is under the inhibitory influence of endogenous episodic release of SRIF (Tannenbaum & Ling 1984) which could have masked the effect of α-FMH on GHRH function. It is thus conceivable that more reliable information on the effect of histaminergic impairment on basal GH secretion in adult rats would have been derived by using a longer sampling time (R. Grilli, V Sibilia, A Torsello, F Pagani, M Guidi, M Luoni, C Netti & E E Müller, unpublished observations). This proposition is compatible with previous reports demonstrating that the inhibitory activity of HA on the spontaneous pulses of GH (Netti et al. 1982) is not removed by SRIF antiserum (Netti et al. 1984).

Reduction of the histaminergic tone by α-FMH increased the GH response to opioid stimulation in both neonatal and adult rats. The modulatory action of HA and opioids on GH secretion is reported to be exerted via an effect on the GHRH pathway (Netti et al. 1984, Murakami et al. 1985); it is therefore conceivable that α-FMH, by removing an inhibitory control of GHRH function, could have magnified the ability of FK 33–824 to stimulate GH release.

Results obtained in young-adult rats after short-term i.c.v. administration of α-FMH are difficult to interpret. One possibility worth considering is that brain HA depletion by α-FMH had induced, after a 3-day treatment, up-regulation of HA receptors (Poulakis & Gertner 1986, Mitsuhashi & Payan 1988), thus allowing re-establishment of central histaminergic tone brought about by the residual transmitter pool. This would explain the inhibition of GHRH mRNA levels and the preservation of SRIF mRNA levels, in view of the reported inability of HA to act on the SRIF system (Netti et al. 1984).

These findings overlap those of Bruno et al. (1993) who reported that short-term systemic administration of α-FMH in adult rats decreased hypothalamic GHRH mRNA levels leaving SRIF mRNA levels unchanged. The fact that we obtained the same results by injecting α-FMH i.c.v. strongly indicates that central and not peripheral HA is involved in the regulation of somatotropic function.

An alternative possibility, i.e. that a GH autoregulation mechanism is responsible for the inhibition of GHRH mRNA levels (Müller 1987), is discounted by the lack of enhanced basal GH levels and ensuing elevated hypothalamic SRIF mRNA levels.

In this context, it is also of interest to recall that short-term impairment of central HA activity affected somatotropic function differently in adult and neonatal rats. This is probably due to the immaturity of the central histaminergic system at birth, since HA levels in the hypothalamus reach adult values only 8–13 days after birth (Subramanian et al. 1981). α-FMH is not effective at depleting hypothalamic HA until postnatal day 9 (Slotkin et al. 1983), suggesting that, in neonatal rats, sustained α-FMH treatment mimics more closely the acute rather than the short-term α-FMH treatment in adult rats. This proposition is also supported by the data showing that neuronal HA and the histaminergic receptor/effector systems in the central nervous system are almost identical ontogenetically (Subramanian et al. 1981).

In summary, our results demonstrate that impairment of histaminergic function facilitates GH release, suggesting that HA exerts an inhibitory effect on GH secretion in both neonatal and young-adult rats. Partial immaturity of the HA system in early postnatal life and a different functional role of the GH-regulatory factors during ontogeny could account for the differences in hypothalamic and pituitary indices of somatotropic function observed in neonatal and adult rats after HA depletion.

Acknowledgements

The authors thank the National Institute of Diabetes, Digestive and Kidney Diseases of the National Institutes of Health for kindly providing the reagents for the GH assay. These studies were supported in part by MURST 40% (to C N) and MURST 60% (to E E M). We thank...
Dr Francesco Pieretti for valuable advice on animal maintenance and treatment.

References


Mitsushashi M & Payan DG 1988 Phorbol ester-mediated desensitization of histamine H1 receptors on a cultured smooth muscle cell line. Life Sciences 43 1433–1440.


