Effect of histamine on the secretion of pro-opiomelanocortin derived peptides in rats

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Abstract. In conscious male rats intracerebroventricular infusion of histamine increased the plasma concentrations of ACTH and β-endorphin immunoreactivity 2.5-fold (P < 0.01). Gel filtration of plasma revealed two peaks of β-endorphin immunoreactivity corresponding to β-endorphin and β-lipotropin. The two fractions increased almost equally in histamine-stimulated animals, whereas most of the circulating β-endorphin immunoreactivity in control animals corresponded to β-endorphin. Central infusion of the H₁-receptor agonist 2-thiazolylethylamine and of the H₂-receptor agonists dimaprit or 4-methylhistamine increased the plasma ACTH and β-endorphin immunoreactivity concentrations 2- and 3-fold, respectively (P < 0.01). Infused intracerebroventricularly, the H₂-receptor antagonists cimetidine or ranitidine prevented the histamine-induced increase in plasma ACTH and β-endorphin immunoreactivity (P < 0.01), whereas the H₁-receptor antagonist mepyramine inhibited the peptide responses by 70% (P < 0.01). Infused intravenously cimetidine or ranitidine inhibited the histamine-induced increase in plasma ACTH by 80% (P < 0.01) and plasma β-endorphin immunoreactivity by 45% (P < 0.05), whereas mepyramine or the other H₁-receptor antagonist SKF-93944 inhibited the ACTH response by 50% (P < 0.05), but had no effect on the β-endorphin immunoreactivity. The results indicate that histamine increases the release of the pro-opiomelanocortin derived peptides ACTH, β-lipotropin and β-endorphin from the anterior pituitary lobe, whereas an effect of histamine on the release of β-endorphin from the neurointermediate lobe is possible. The effect of histamine seems primarily mediated by H₂-receptors, whereas H₁-receptors appear to play a minor role.

Histamine (HA) is a likely candidate as a neurotransmitter in the central nervous system (Prell & Green 1986). The highest concentration of the amine as well as histamine H₁- and H₂-receptors has been found in the hypothalamus, and recent immunohistochemical studies have demonstrated histaminergic neurons in that area. It has been proposed that HA, besides other hypothalamic actions, participates in the neuroendocrine regulation of pituitary hormone secretion (Weiner & Ganong 1978). HA stimulates ACTH secretion when infused intracerebroventricularly (icv) in dogs (Rudolph et al. 1979) and systemically in rats (Morita & Koyama 1979; Reilly & Sigg 1982), and HA infused icv in rats increases basal and stress-induced corticosterone secretion (Bugajski & Gadek 1983).

Although the effect of HA on ACTH secretion has been intensively studied, the effect of the amine on the secretion of β-endorphin (β-END) and β-lipotropin (β-LPH) has not been investigated. The three peptides as well as α-MSH are processed from the same precursor molecule, POMC (Mains et al. 1977; Nakanishi et al. 1979).
In the rat, the anterior pituitary lobe releases ACTH, β-LPH, and β-END, whereas the neuro-intermediate lobe releases β-END and α-MSH (Imura et al. 1982; Mains & Eipper 1981; Young et al. 1986). Since the POMC-derived peptides seem to be secreted synchronously (Guillemín et al. 1977), it is possible that HA besides its ACTH-releasing action may affect the secretion of β-END and β-LPH. To investigate this possibility, we studied the effect of HA infused icv on plasma concentrations of ACTH and β-END immunoreactivity (β-ENDir) in male rats. In addition, we studied the involvement of H₁ and H₂-receptors in the mediation of this response and the effect of HA on the chromatographic profile of β-ENDir in plasma.

Material and Methods

Animals

Male rats of the Wistar strain (275–325 g) bred at the Panum Institute were housed under controlled conditions of temperature (22 ± 1°C), lighting (lights on 06.00–18.00 h daily), and humidity. The rats had free access to laboratory chow and tap water.

Six to seven days before the experiment, rats were provided with a permanent metal cannula in a lateral ventricle of the brain for intracerebroventricular (icv) infusions. The tip of the cannula was positioned according to the following coordinates: 1.5 mm lateral from the bregma on the coronal suture of the skull, 4.5 mm below the surface of the skull. The cannula was fixed to the skull with skrews and dental cement. Three to four days prior to experimentation some of the rats were also provided with a silastic catheter in a femoral artery for intra-arterial (ia) infusions. The catheter was filled with saline containing heparine (330 kIU/l) and led sc to the neck where it was exteriorized and sealed. All surgical procedures were performed during pentobarbital anesthesia (60 mg/kg, ip).

Drugs

The drugs used were HA (0.27 μmol as free base icv; Sigma Chemicals, St. Louis, MO), the H₁-receptor agonist 2-thiazolyethylamine (2-TEA; 1.09 μmol icv), the H₂-receptor agonists 4-methylhistamine (4-MeHA; 0.79 μmol icv), and dimaprit (DIM; 1.86 μmol icv). All agonists were from Smith, Kline & French, Welwyn Garden, England. The doses used were calculated from the activity of the agonists on H₁ or H₂-receptors relative to that of HA in vitro (Ganellin 1982). The H₁-receptor antagonists were mepyramine (MEP; 0.35 μmol icv and 3.50 μmol ia; DAK, Copenhagen, Denmark) and SKF-93944 (SKF; 3.39 μmol ia; Smith, Kline & French), and the H₂-receptor antagonists cimetidine (CIM; 0.40 μmol icv and 7.94 μmol ia; Smith, Kline & French) and ranitidine (RAN; 0.40 μmol icv and 7.96 μmol ia; Glaxo Inc, Ware, England). Drugs were dissolved in saline or in saline acidified with 0.1 N HCl and infused at pH 5.4–6.0. Saline at the same pH was infused in control experiments.

Experimental procedures

At the day of the experiment the icv cannula and the femoral catheter were extended by silastic tubings permitting infusion of test substances without disturbing the animal. The rats were then left undisturbed in individual cages in a quiet room for an adaptation period of at least 90 min before decapitation.

Saline or HA receptor antagonists were infused icv (5 μl; 2 μl/min) or ia (1 ml) at time −5 min followed by an icv infusion of saline, HA or HA receptor agonists (5 μl) at time 0 min. The animals (8–12 in each group) were decapitated 15 min later.

Groups of non-pretreated animals (8 in each group) were exposed to either restraint stress (the animal was fixed on its back for 5 min) or ether stress (the animal was exposed to ether vapour for 5 min).

Trunk blood was collected into tubes containing 500 IU heparine. The tubes were centrifuged at 4°C and plasma was stored at −20°C until assayed for ACTH and β-ENDir.

Radioimmunoassay of ACTH and β-ENDir

ACTH was measured in unextracted plasma by RIA. Antiserum, raised in rabbits to porcine ACTH, was obtained from the National Pituitary Agency, NIADDK, NIH. The antiserum is directed towards the 1–24 sequence of ACTH and has less than 0.4% cross-reactivity with α-MSH and β-MSH. The antiserum was used in a final dilution of 1:80 000. Synthetic human ACTH (1–39), used for iodination and standards, was obtained by the National Pituitary Agency, NIH. ¹²⁵I-ACTH was prepared by the chloramine-T method and the labelled hormone was purified on a Sephadex G-50 column (Pharmacia Fine Chemicals, Uppsala, Sweden). The specific activity of ¹²⁵I-ACTH was 4.9 MBq/μg as determined by self displacement.

On the first day of the assay 3000 cpm of ¹²⁵I-ACTH in a volume of 100 μl and 100 μl antiserum was incubated for 18 h at 4°C with 100 μl of standards or unknown samples mixed with 200 μl assay buffer (0.02 mol/l veronal buffer, pH 8.6, containing 0.5% human HSA, 400 KIU/ml aprotinin, 0.01% merthiolat, and 0.4% mercaptoethanol) in a final volume of 500 μl. Following incubation bound and free ¹²⁵I-ACTH were separated using dextran-coated charcoal. Dilution of rat plasma and of the standard gave parallel binding curves. The least detectable quantity of ACTH was 1 pmol/l plasma and half maximal binding of tracer was obtained when the reaction tubes contained an amount
of ACTH corresponding to 52 pmol/l plasma. The intra- and inter-assay coefficient of variation was 4 and 5%, respectively. The concentration in resting, non-stressed rats was 46 ± 10 pmol/l (mean ± SD; N = 17).

β-ENDir was extracted from plasma using silicic acid, 100 MESH (Mallinckrodt, St. Louis, MO), added to plasma (25 g/l). After washing with distilled water and ether, β-ENDir was desorbed with a mixture of aceton and acetic acid 1% (40:60, v/v) and the solvent was evaporated by a gentle steam of nitrogen. The remnant was reconstituted in assay buffer (0.05 mmol/l phosphate buffer, pH 7.4, containing 0.9% saline, 1% HSA, 0.5% mercaptoethanol, 500 KIU/ml aprotinin, and 0.1% Tween 20 (Merck-Schuchardt, Munich, FRG).

Extracted β-ENDir was quantified by RIA. The antiserum against β-END (No. 5422), kindly provided by Dr D. Marshak, Houston, TX, cross-reacts 100% with β-LPH but shows no cross-reaction with α-END, γ-END, ACTH 1–24, γ-MSH, dynorphin 1–13, met-enkephalin, or leu-enkephalin. β-END 1–27 cross-reacted less than 1% on molar basis. The antiserum was used in a final dilution of 1:210 000. Synthetic human β-END, used for standards and iodination, was obtained from Peninsula Laboratories, CA. 125I-β-END was prepared by the stoichiometric chloramine-T method and the labelled hormone was purified on a Sephadex G-50 superfine column (Pharmacia Fine Chemicals). The specific activity of 125I-β-END was 1.4 MBq/µg as determined by self displacement.

On the first day of the assay, 200 µl of reconstituted sample was mixed with an equal volume of the antisem and incubated for 72 h at 4°C. Thereafter, 200 µl 125I-β-END was added and the incubation was continued for another 24 h at 4°C. Bound and free 125I-β-END were separated using dextran-coated charcoal. Recovery of 100 pg human β-END and human β-LPH (Bioflex, Ozone, NY) added to plasma before extraction was 58 ± 6% and 62 ± 14%, respectively (mean ± SD; N = 21). Dilution of rat plasma and of the standard gave parallel binding curves. Detection limit was 4 pmol/l rat plasma and half maximal binding of tracer was obtained when the reaction tubes contained an amount of β-END corresponding to 100 pmol/l plasma. The intra- and inter-assay coefficient of variation was 8 and 15%, respectively.

**Gel filtration**

Plasma extracts and standards were applied on a 1.5 × 52 cm Sephadex G-50 superfine column (Pharmacia Fine Chemicals). The column was equilibrated and eluted with 1% acetic acid containing 0.02% HSA (Behringwerke, FRG). Fractions of 2 ml were collected from sample and standard runs, lyophilized, and reconstituted in 300 µl assay buffer of which 200 µl was used in the assay. The column was calibrated with blue dextran as void volume marker (V₀), human β-END and β-LPH, and Na₂¹²⁵I as a marker of the total volume (Vₜ). Blue dextran and Na₂¹²⁵I were added to sample or standard in each run to determine the fractional elution volume (Vₐ) of immunoreactive materials. Pools of approximately 5 ml of plasma from rats infused icv with saline (N = 4) or 30 µg of HA (N = 4) were applied to the column.

**Statistical procedures**

Results are presented as the mean ± sem. Statistical evaluation was performed by one-way analysis of variance followed by Duncan’s test for multiple comparisons. The limit of significance was P < 0.05.

**Fig. 1.**

Effect of the H₁ receptor antagonists mepyramine (MEP) or SKF-93944 (SKF) or the H₂ receptor antagonists cimetidine (CIM) or ranitidine (RAN) on basal (upper panel) or histamine-stimulated (lower panel) secretion of ACTH in male rats (N = 8–12 in each group). The antagonists were infused icv or ia 5 min before histamine or saline, which was infused icv 15 min before decapitation of the animals. The doses of the antagonists are given in the text. The dashed line in the first column of the lower panel represent the basal level of ACTH. Each bar graph represent the mean (± sem).

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Effect of histamine (HA), the H₁-receptor agonist 2-thiazolylethylamine (2-TEA) or the H₂-receptor agonists dimaprit (DIM) and 4-methylhistamine (4-MeHA) on the release of ACTH and β-ENDir in male rats (N = 8 in each group). Histamine or the agonists were infused icv 15 min before decapitation. Each bar graph represent the mean (±SEM).

Results

Effect of histamine on the release of ACTH
Plasma ACTH concentration in saline-infused control animals was 51 ± 2 pmol/l (Fig. 1). Infusion of the H₁- or H₂-receptor antagonists had no effect on basal plasma ACTH concentration (Fig. 1).

Central infusion of HA (0.27 µmol) increased the ACTH concentration 2.5-fold to 113 ± 5 pmol/l (P < 0.01; Fig. 1). Prior icv infusion of the H₂-receptor antagonists CIM or RAN prevented the response (50 ± 4 and 49 ± 4 pmol/l, respectively (P < 0.01), whereas the H₁-receptor antagonist MEP inhibited the effect of HA by 70% (68 ± 8 pmol/l; P < 0.01). Systemic infusion of CIM or RAN reduced the ACTH response to HA by 80% (58 ± 8 and 66 ± 9 pmol/l, respectively, P < 0.01), whereas ia infusion of MEP or the other H₁-receptor antagonist SKF-91488 diminished the response to HA by 50% (77 ± 3 and 83 ± 7 pmol/l, respectively; P < 0.05).

Central infusion of the H₁-receptor agonist 2-TEA (1.09 µmol) increased plasma ACTH concentration almost 2-fold (93 ± 9 pmol/l; P < 0.01; Fig. 2), whereas the H₂-receptor agonists 4-MeHA (0.79 µmol) and DIM (1.86 µmol) caused a more than 3-fold increase in the plasma ACTH level (139 ± 8 and 148 ± 13 pmol/l, respectively, P < 0.01; Fig. 2).

Effect of histamine on the release of β-ENDir
Plasma concentration of β-ENDir in saline infused control animals was 27 ± 3 pmol/l (Fig. 3). Intra-cerebroventricular or ia infusion of the H₁- or H₂-receptor antagonists had no effect on basal secretion of β-ENDir.

Central infusion of HA (0.27 µmol) caused a 2.5-fold increase in the plasma β-ENDir level, from 27 ± 3 to 74 ± 5 pmol/l (P < 0.01; Fig. 3). CIM or RAN infused icv abolished the response (25 ± 9 and 28 ± 8 pmol/l, respectively; P < 0.01), whereas icv infusion of MEP attenuated the response to HA by 80% (37 ± 11 pmol/l, P < 0.01). Systemic infusion of CIM or RAN inhibited the β-ENDir response to HA by approximately 45% (54 ± 4 and 51 ± 5 pmol/l, respectively; P < 0.05), whereas ia infusion of the H₁-receptor antagonists MEP or SKF-91488 had no effect (83 ± 8 and 78 ± 12 pmol/l, respectively).

Central infusion of the H₂-agonists 4-MeHA and DIM increased the plasma β-ENDir 3-fold (87 ± 9 and 83 ± 5 pmol/l, respectively; P < 0.01; Fig. 2), whereas ia infusion of the H₁-receptor
agonist 2-TEA caused only a 2-fold increase (53 ± 6 pmol/l; \( P < 0.01 \); Fig. 2). There was a highly significant correlation between the plasma concentrations of \( \beta \)-ENDir and ACTH following icv infusion of saline, 2-TEA, HA, DIM, and 4-MeHA (\( r = 0.9876, P < 0.01 \)).

Restraint as well as ether stress increased the plasma concentration of ACTH (157 ± 10 and 109 ± 6 pmol/l, respectively) and \( \beta \)-ENDir (175 ± 15 and 152 ± 15 pmol/l, respectively).

Gel filtration of plasma from control rats (Fig. 4) revealed one peak co-eluting with synthetic \( \beta \)-END and a small peak co-eluting with synthetic \( \beta \)-LPH. In animals infused icv with HA two large peaks of a similar size appeared, co-eluting with synthetic \( \beta \)-END and synthetic \( \beta \)-END and synthetic \( \beta \)-LPH.

Sephadex G-50 gel filtration profiles of \( \beta \)-ENDir in extracts of rat plasma after icv infusion of NaCl (control) or histamine (HA) (\( N = 4 \)). Elution positions are referred to by the fractional elution volume \( [K_d=(V_r-V_0)/V_r, where V_r \text{ is the elution volume of the peak in question, } V_0 \text{ the void volume, and } V_r \text{ the available inner volume determined as the difference between the elution volume of NaCl and the void volume. The } K_d \text{ of human } \beta \text{-LPH and } V_r \text{ is indicated. } \bullet \text{—-\bullet } \text{HA icv; } \circ \text{---\circ } \text{NaCl icv.}

**Fig. 4.**

Effect of the \( H_1 \)-receptor antagonists mepyramine (MEP) or SKF-93944 (SKF) or the \( H_2 \)-receptor antagonists cimetidine (CIM) or ranitidine (RAN) on basal (upper panel) or histamine-stimulated (lower panel) secretion of \( \beta \)-ENDir in male rats (\( N = 8 - 12 \) in each group). For further details, see Fig. 1.

**Discussion**

We found that central infusion of HA to male rats stimulated the secretion of the POMC-derived peptides, ACTH and \( \beta \)-ENDir. The effect of HA was mediated through activation of \( H_1 \) as well as \( H_2 \)-receptors, since the HA-induced stimulation of ACTH and \( \beta \)-ENDir was mimicked by \( H_1 \) and \( H_2 \)-receptor agonists and inhibited by \( H_1 \) and \( H_2 \)-receptor antagonists. However, the effect mediated via \( H_2 \)-receptors seemed to predominate, since the stimulatory action of the \( H_2 \)-receptor agonists and the inhibitory action of the \( H_2 \)-receptor antagonists were more pronounced than the corresponding actions of the \( H_1 \)-receptor agonists or antagonists.

The stimulatory effect of HA on ACTH secretion obtained in the present investigation is in accordance with previous studies, in which HA
infused icv or systemically (iv or ip) to rats or dogs stimulated the pituitary-adrenal axis. However, different results have been obtained concerning the HA receptors involved in the mediation of the response. In conscious rats both H1- and H2-receptors mediated the response (Bugajski & Gadek 1983; Morita & Koyama 1979; Reilly & Sigg 1982), whereas in ether anesthetized rats only H1-receptors were involved (Seltzer & Donoso 1982). In pentobarbital anesthetized dogs stimulation of H1-receptors augmented ACTH secretion, whereas stimulation of H2-receptors inhibited the secretion (Rudolph et al. 1979). These discrepancies in receptor mediation of the response may partly be explained by species differences, the influence of anesthetics, and use of different administration routes, and doses of the compounds.

HA infused icv increased plasma ß-ENDir. Gel filtration of plasma from HA-stimulated rats showed that ß-LPH and ß-END were released in similar quantities. In contrast, most of the circulating ß-ENDir in plasma from control animals was ß-END, which is in accordance with previous results (Young et al. 1986). Since ß-LPH is exclusively produced in the anterior lobe, whereas ß-END is produced both in the anterior and the neurointermediate lobe (Imura & Eipper 1981; Young et al. 1986), the results indicate that HA stimulates the release of ß-ENDir from the anterior lobe of the pituitary, but they do not clarify whether HA effects the release of ß-ENDir from the neurointermediate lobe.

The HA-induced increase in plasma ß-ENDir concentration was parallel to that of ACTH and a highly significant correlation was found between plasma levels of ACTH and ß-ENDir following icv infusion of HA and the agonists. Furthermore, central infusion of the antagonists reduced the HA-stimulated secretion of the two peptides in a similar manner. These results indicate that ACTH and ß-ENDir are concomitantly released from the anterior pituitary lobe following stimulation with HA. The finding is in accordance with the previous suggestion of a synchronous release of ACTH and ß-ENDir (Guillemín et al. 1977). However, differences in the plasma levels of ACTH and ß-ENDir were obtained after some of the experiments. Thus, systemic infusion of the H1-receptor antagonists inhibited HA-stimulated ACTH release, but had no effect on the HA-stimulated ß-ENDir release. At present this discrepancy is difficult to explain. ß-ENDir released by HA may originate from both the anterior and the neurointermediate lobe, whereas ACTH is released only from the anterior lobe. Systemic infusion of the H1-receptor antagonists may block the effect of HA on ACTH and ß-ENDir secretion from the anterior lobe, but not that on ß-ENDir secretion from the neurointermediate lobe, leaving the plasma ß-ENDir concentration unaffected.

The increase in plasma ACTH concentration followed restraint and ether stress were almost identical to the HA-induced increase. However, the ß-ENDir responses to restraint and ether stress were approximately 2-fold higher than that induced by HA. Since stress stimulates the release of ß-ENDir from the anterior as well as the neurointermediate lobe (Berkenbosch et al. 1984), it may be suggested that HA primarily affects the release of ß-ENDir from the anterior lobe.

It has previously been found that HA was unable to exert a direct stimulatory effect on the release of ACTH or ß-ENDir from incubated pituitary glands (Fehm et al. 1980; Hashimoto et al. 1979; Vermes et al. 1980). In the present study we have shown that the inhibitory effect of HA receptor antagonists was more pronounced following central than systemic administration. Taken together, these observations indicate that the effect of HA occurs at a suprapituitary level by an action on hypothalamic factors regulating the secretion of the POMC-derived peptides. It has been suggested that catecholamines via activation of ß-adrenergic receptors are involved in the adrenocortical response to HA (Bugajski & Gadek 1984). The effect of HA may also be transmitted via an effect on CRH, which potently stimulates the release of ACTH and ß-ENDir (Przewlocki et al. 1979; Rivier et al. 1982, 1984; Vermes et al. 1980). However, in previous studies HA had no effect on the release of bioassayable CRF from hypothalamic tissue in vitro (Fehm et al. 1980; Hillhouse et al. 1975). AVP may be involved in the mediation of the response to HA, since AVP is released by HA (Dogterom et al. 1976; Mens & van Wimersma Greidanus 1982), and since AVP stimulates the release of ACTH and ß-ENDir (Rivier et al. 1984; Vermes et al. 1980).

In summary, HA infused centrally stimulates the release of the POMC-derived peptides ACTH,
β-END, and β-LPH from the pituitary of male rats. This effect is caused by activation of H₁ and predominantly H₂ receptors, presumably located in the hypothalamus.

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