EXPERIMENTAL STUDY

Effect of blockade of postsynaptic H1 or H2 receptors or activation of presynaptic H3 receptors on catecholamine-induced stimulation of ACTH and prolactin secretion

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Abstract

The effect of inhibition of the neuronal histaminergic system by blockade of postsynaptic H1 or H2 receptors or activation of presynaptic H3 autoreceptors on the ACTH and prolactin responses to the catecholamines epinephrine and norepinephrine was investigated in conscious male rats. Intracerebroventricular infusion of epinephrine and norepinephrine stimulated ACTH and prolactin secretion. Prior intracerebroventricular infusion of the H1 receptor antagonist, mepyramine, or the H2 receptor antagonist, cimetidine, had no effect on the ACTH response to epinephrine or norepinephrine, while these responses were inhibited by pretreatment with the H3 receptor agonist, imetit. The prolactin response to norepinephrine was significantly inhibited by pretreatment with mepyramine, cimetidine or imetit whereas the three histaminergic compounds had no effect on the prolactin response to epinephrine. The findings suggest that the histaminergic system exerts a mediating or permissive action on the norepinephrine-induced stimulation of prolactin secretion, whereas an intact histaminergic system may not be required for catecholamines to stimulate ACTH secretion. The inhibitory effect of imetit on catecholamine-induced release of ACTH may be due to an activation of H3 receptors located presynaptically on non-histaminergic neurons, e.g. aminergic neurons. The study further indicates an important role of histamine in the neuroendocrine regulation of prolactin secretion.

European Journal of Endocrinology 142 637–641

Introduction

Originating from neurons in the posterior hypothalamus, histamine (HA) acts as a neurotransmitter in the brain (1–5). Among its several actions, it has been established that HA participates in the neuroendocrine regulation of adrenocorticotropin (ACTH) and prolactin (PRL) secretion and that this regulatory effect is indirect and exerted in the hypothalamus (6). The effect of HA on ACTH secretion is caused by an effect on corticotropin-releasing hormone neurons and vasopressin (AVP) neurons in the paraventricular nucleus (7–9) – probably via an interaction with prostaglandins (10). The effect of HA on PRL secretion seems to involve AVP and serotonin (11, 12), while the involvement of dopamine is in question (13–16).

We have recently found that specific blockade of $\alpha 1$ and $\beta 1$ adrenergic receptors inhibited the HA-induced PRL response (17), suggesting that HA interacts with catecholamines via these receptor subtypes in the

regulation of PRL secretion. In contrast, we found that the ACTH response to HA was not affected by blockade of adrenergic receptors.

The catecholamines, epinephrine (E) and norepinephrine (NE), which originate in perikarya in the brain stem, participate in the neuroendocrine regulation of ACTH and PRL secretion (18, 19) predominantly exerting a stimulatory effect on secretion of these two hormones (18–24).

Several studies indicate that histaminergic and catecholaminergic neurons are both involved in the mediation of the stress-induced release of ACTH and PRL (21, 25–28). Furthermore, immunohistochemical studies have shown that histaminergic nerve fibers from the posterior hypothalamus, as well as catecholaminergic nerve fibers from the brain stem project to the paraventricular nucleus and median eminence and that catecholaminergic afferents from the brain stem innervate histaminergic neurons in the posterior hypothalamus (3–5, 18, 19). Therefore, it is possible that the

activity of histaminergic neurons may influence the effect of the catecholamines E and NE on ACTH and PRL secretion.

In order to investigate this possibility, we studied whether inhibition of histaminergic neuronal transmission by blockade of postsynaptic H1 or H2 receptors or activation of presynaptic H3 receptors affected the ACTH and PRL responses to E and NE in conscious male rats.

Materials and methods

Animals

Male rats of the Wistar strain (275–325 g) bred at the Panum Institute were housed under controlled conditions of temperature ($22 \pm 1^\circ\text{C}$), humidity (40–50%) and lighting (lights on between 0600 and 1800 h daily). The rats had free access to laboratory chow and tap water. The well-being of the animals was secured in concordance with Danish national rules set forth by the Ministry of Justice.

Compounds

The following histaminergic compounds were used: the H1 receptor antagonist, mepyramine maleate (MEP; a gift from DAK, Copenhagen, Denmark), the H2 receptor antagonist, cimetidine 2HCl (CIM; a gift from GEA, Copenhagen, Denmark) and the H3 receptor agonist, imetit 2HBr (IME; a gift from Professor Timmerman, Free University, Amsterdam, The Netherlands). The following catecholaminergic compounds were used: the $\alpha + \beta$ receptor agonist, epinephrine bitartrate (E; Research Biochemical International (RBI), Natick, MA, USA), and the $\alpha + \beta$ 1 receptor agonist, norepinephrine bitartrate (NE; RBI). All compounds were dissolved in saline except CIM, which was dissolved in saline acidified with 0.1 M HCl and adjusted with 0.1 M NaOH to pH 7.5. All compounds were administered intracerebroventricularly (icv) except IME, which was injected intraperitoneally (ip). The catecholaminergic compounds E and NE were administered in an equimolar dose of 5 mmol icv. The doses of MEP (350 nmol icv) and CIM (400 nmol icv) have previously been found to inhibit HA-induced hormone release (26, 29) and the dose of IME (2 mg/kg ip) has been found to inhibit stress-induced hormone release (30).

Experimental procedures

Approximately one week prior to experimentation, a permanent cannula was implanted in a lateral ventricle of the brain during pentobarbital anesthesia (60 mg/kg ip) as previously described (26). On the day of the experiment, the cannula was extended by silastic tubing filled with solutions of saline or H1 or H2 receptor

antagonist and solutions of E, NE or saline. This permitted icv infusion of test substances without disturbing the rats. All experiments were performed between 1000 and 1400 h after the rats had adapted in the laboratory for 90–120 min in individual cages.

MEP and CIM were infused icv in a volume of 5 μl at time -20 min. IME was injected ip in a volume of 1 ml at time -180 min. E and NE were infused icv in 5 μl at -15 min. Administration of saline served as control. The animals were decapitated at 0 min and blood was collected from the trunk. The number of animals in each group ranged from 6 to 10.

Hormone analysis

Blood was collected in polyethylene tubes containing 100 μl 0.5 mol/l ethylenediaminetetraacetic acid (EDTA) and 50 μl aprotinin (Trasylol 20 000 KIU/ml; Bayer, Leverkusen, Germany). The blood samples were centrifuged at 4°C and plasma was stored at -20°C until analyzed for ACTH and PRL by specific radioimmunoassays (29, 31). The hormone determinations were performed in unextracted plasma. The ACTH antiserum (generously provided by the National Pituitary Agency, NIH, Bethesda, MD, USA) does not cross-react with β -endorphin or β -lipotropin and has less than 0.4% cross-reactivity with α -melanocyte stimulating hormone (α -MSH) and β -MSH. Synthetic human ACTH(1–39) (generously provided by the National Pituitary Agency, NIH) served as reference preparation and was used for iodination. The least detectable quantity of ACTH was 1 pmol/l plasma and the intra- and interassay coefficients of variation were 4 and 5% respectively. PRL was determined by RIA as outlined by the NHPP (Bethesda, MD, USA) using PRL-RP-3 as standard. The minimal detectable quantity was 0.4 $\mu\text{g/l}$. Intra- and interassay coefficients of variation were 3.6% and 9.9% respectively.

Statistical procedures

Results are presented as the mean $\pm$ S.E.M. and evaluated by one way analysis of variance, followed by Duncan's test for multiple comparisons when appropriate. The limit of significance was $P < 0.05$.

Results

Effect of H1 or H2 receptor blockade or H3 receptor activation on basal ACTH and PRL secretion

Basal secretion of ACTH or PRL was not affected by treatment with the H1 receptor antagonist, MEP, the H2 receptor antagonist, CIM, or the H3 receptor agonist, IME (Table 1).

Table 1 Effect of the H1 receptor antagonist, MEP, the H2 receptor antagonist, CIM, or the H3 receptor agonist, IME, on basal secretion of ACTH or PRL in male rats. The data represent the mean $\pm$ S.E.M. of 6–8 animals.

	Saline	MEP	CIM	IME
ACTH (pmol/l)	64 $\pm$ 6	59 $\pm$ 11	67 $\pm$ 18	63 $\pm$ 9
PRL (μ g/l)	6 $\pm$ 2	6 $\pm$ 2	4 $\pm$ 2	9 $\pm$ 3

Effect of H1 or H2 receptor blockade or H3 receptor activation on E- or NE-stimulated ACTH and PRL secretion

ACTH secretion was stimulated 2.5-fold by E ($P < 0.01$) and 2-fold by NE ($P < 0.01$) (Fig. 1) whereas PRL secretion was increased 4-fold by E ($P < 0.01$) and 5-fold by NE ($P < 0.01$) (Fig. 2).

The ACTH response to E or NE was not affected by pretreatment with MEP or CIM. However, pretreatment with the H3 receptor agonist, IME, reduced the ACTH response to E and NE by about 75% ($P < 0.01$; $P < 0.05$) (Fig. 1).

The PRL response to E was not affected by MEP, CIM or IME (Fig. 2). In contrast, the PRL response to NE was almost prevented by MEP, CIM and IME ($P < 0.01$) (Fig. 2).

Discussion

The secretion of ACTH and PRL was stimulated by equimolar doses of the $\alpha + \beta$ receptor agonist, E and the $\alpha + \beta 1$ receptor agonist, NE. These results are in accordance with previous studies (18–24) showing that catecholamines play a predominant stimulatory role in the neuroendocrine regulation of ACTH and PRL secretion.

Pretreatment with the postsynaptic H1 or H2 receptor antagonists MEP or CIM had no effect on the ACTH response to E or NE. This suggests that the histaminergic system does not participate as a mediating or permissive factor in the catecholamine-induced

release of ACTH. In contrast, pretreatment with the presynaptic H3 receptor agonist IME inhibited the ACTH response to E and NE. We have previously found that IME as well as other H3 receptor agonists inhibited the ACTH response to different stress stimuli (30), an effect that was prevented by concomitant administration of an H3 receptor antagonist. The difference between the lack of effect of the H1 and H2 receptor antagonists and the inhibitory effect of the H3 receptor agonist is difficult to explain, since similar actions were to be expected. However, although IME does cross the blood–brain barrier, IME and MEP/CIM may have different sites of action due to the different administration routes (IME ip vs MEP/CIM icv). Since we have previously found similar inhibitory effects on stimulated ACTH secretion of H1 and H2 receptor antagonists administered centrally and of H3 receptor agonists administered systemically (30, 32, 33), another explanation is more likely. It has been reported that H3 receptors, in addition to their presynaptic localization on histaminergic neurons are also located presynaptically on other aminergic or cholinergic neurons (34–36). The effect of IME found in this study may, therefore, be due to a presynaptic effect of the compound on non-histaminergic neurons (e.g. catecholaminergic neurons) which activate the hypothalamic–pituitary–adrenal axis.

The catecholamines E and NE significantly increased PRL secretion in accordance with the generally stimulatory role of catecholamines in the central regulation of PRL secretion (23, 24, 37). The stimulatory effect of E on PRL secretion was not affected by any of the histaminergic compounds. In contrast, blockade of H1 or H2 receptors as well as activation of H3 receptors almost prevented the PRL response to NE. This suggests that the histaminergic system mediates the effect of NE on PRL secretion or that intact activity of the histaminergic system is required for NE to stimulate PRL secretion – at least in the dose used here. The similar effects of the H1 and H2 receptor antagonists and the H3 receptor agonist on PRL secretion suggest that the effect of IME is caused by activation of presynaptic receptors located on histaminergic neurons,

Figure 1 Effect of the H1 receptor antagonist MEP, the H2 receptor antagonist CIM or the H3 receptor agonist IME on secretion of ACTH in male rats stimulated by E or NE. The H1 and H2 receptor antagonists were infused icv at time –20 min, the H3 receptor agonist was infused ip at –180 min, E or NE were infused icv at –15 min and the animals were decapitated at 0 min. The data represent the mean $\pm$ S.E.M. of 8–10 rats in each group. ## $P < 0.01$ vs control (Cont; Sa, saline); * $P < 0.05$ vs NE+Sa; ** $P < 0.01$ vs E+Sa.

Figure 2 Effect of the H1 receptor antagonist MEP, the H2 receptor antagonist CIM or the H3 receptor agonist IME on secretion of PRL in male rats stimulated by E or NE. The H1 and H2 receptor antagonists were infused icv at time -20 min, the H3 receptor agonist was infused ip at -180 min, E or NE were infused icv at -15 min and the animals were decapitated at 0 min. The data represent the mean $\pm$ S.E.M. of 8–10 rats in each group. ## P <0.01 vs control (Cont; Sa, saline); ** P <0.01 vs NE+Sa.

although an additional effect of the compound exerted on other aminergic neurons cannot be excluded.

The discrepancy between the inhibitory effect of the histaminergic compounds on NE-stimulated PRL secretion and their lack of effect on E-induced stimulation is difficult to explain as NE and E affect α - as well as β -adrenergic receptors and using the same infusion routes may be expected to exert their effect at adrenergic receptors within the same brain areas.

We have recently reported that blockade of α_1 or β_1 adrenoreceptors inhibited the PRL response to central administration of HA whereas the ACTH response to HA remained unaffected during central pretreatment with selective or non-selective α or β adrenoreceptor antagonists (17). Compared with the present study, we suggest that catecholamines and histamine interact in the central regulation of PRL secretion whereas the transmitters do not exert synergistic actions in the central regulation of ACTH secretion.

In summary, we found that the histaminergic system may play a mediating or permissive role in NE-induced stimulation of PRL secretion – but not in E-induced stimulation of PRL secretion – whereas an intact histaminergic system does not seem to be required for catecholamine-induced stimulation of ACTH secretion. However, activation of presynaptic H3 receptors – probably located on other aminergic neurons – may reduce the ACTH response to E and NE. The findings consolidate the role of histamine in the neuroendocrine regulation of prolactin secretion.

Acknowledgements

This study was supported by grants from the Danish Medical Research Council, Ib Henriksen's Foundation, the Velux Foundation, Jacob and Olga Madsen's Foundation, P Carl Petersen's Foundation, the Danish Hospital Foundation for Medical Research, Regions of Copenhagen, the Faeroe Islands and Greenland, NOVO's Foundation, Nordisk Forsknings Komité, Poul M and Birthe Christiansen's Foundation, Gerda and Aage Haensch's Foundation, and the Foundation for the

Advancement of Medical Research. Part of the study was supported by the Commission of the European Communities (contract: CEE BMH1-CT 92-1087).

We thank Elsa Larsen and Jytte Oxbøl for skilled technical assistance. The materials for RIA of prolactin and ACTH were kindly provided by the National Hormone and Pituitary Program of the NIDDK, Bethesda, MD, USA.

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Received 18 October 1999

Accepted 17 February 2000