Effect of Endothelin-1 in Man—Impact on Basal and Stimulated Concentrations of Luteinizing Hormone, Follicle-Stimulating Hormone, Thyrotropin, Growth Hormone, Corticotropin, and Prolactin With and Without Pretreatment With Nifedipine

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In healthy men, intravenous (IV) endothelin-1 suppresses the growth hormone (GH)-releasing hormone (GHRH)-stimulated increase in GH and prolactin (PRL) and augments corticotropin (ACTH)-releasing factor (CRF)-stimulated secretion of ACTH. Since some actions of endothelin-1 on pituitary function in vitro are antagonized by calcium channel antagonists, we have studied the effect of pretreatment with oral nifedipine (10 mg, given before infusion of endothelin-1 or vehicle) on basal and stimulated concentrations of pituitary hormones in a group of healthy men (N = 6). The augmentative effect of endothelin-1 on CRF-induced ACTH secretion ($P < .05$) was counteracted by pretreatment with nidefipine. Pretreatment with nifedipine further inhibited ($P < .01$) the GHRH-induced increase in plasma concentrations of GH ($P < .05$), which, in keeping with previous data, had already been reduced by IV endothelin-1 alone ($P < .05$). Thus, both endothelin-1 and nifedipine influence pituitary hormone secretion in healthy man. However, nifedipine does not ubiquitously counteract the effects of endothelin-1 since it enhances some of its actions on the pituitary and diminishes others. Endothelin-1 may therefore influence pituitary function by mechanisms other than activation of calcium channels alone.

IN ADENOHYPOPHYSEAL cells, endothelin-1 stimulates phosphatidylinositol hydrolysis and mobilizes intracellular calcium,[1] suggesting a potential role of endothelin-1 in stimulus-secretion coupling. In keeping with these observations, we have demonstrated[2] that endothelin-1 exerts a suppressive action on stimulated plasma concentrations of prolactin (PRL) and growth hormone (GH) but stimulates the release of corticotropin (ACTH) in healthy man. Since not only the peptide's vasoconstrictor effect[3] but also the increase in pituitary glucose oxidation induced by endothelin appears to depend on dihydropyridine-sensitive calcium channels,[4] we have investigated the effect of endothelin-1 on basal and stimulated pituitary hormone secretion in healthy men with and without pretreatment with the calcium channel antagonist, nifedipine.

SUBJECTS AND METHODS

Six non-obese, healthy male volunteers aged 25 to 29 years took part in this study. The purpose and potential risks of the study protocol, which had been approved by the local ethics committee, were carefully explained to each participant, and written consent was obtained. No medication was permitted for at least 6 weeks before the study. On the day of experiments, consumption of food and liquid was discontinued at midnight. From 7 AM on, the men remained in the supine position. Indwelling catheters were inserted into each antecubital vein, one for infusion and the other for blood sampling. The following four experiments were performed in random sequence at least 1 week apart.

Control Experiment (Experiment 1)

Following a resting period of 2 hours, each volunteer received an infusion of vehicle (Haemaccel; Behringwerke, Marburg, Germany). Thirty minutes after the beginning of this infusion, each volunteer received an intravenous (IV) bolus dose of 100 μg luteinizing hormone–releasing hormone (LHRH Relefact; Hoechst, Frankfurt/Main, Germany), 400 μg thyrotropin-releasing hormone ([TRH] Relefact; Hoechst), 100 μg human corticotropin-releasing factor ([hCRF] Corticobiss; Chemie Bissendorf, Bissendorf, Germany), and 100 μg GH-releasing hormone ([GHRH] Somatobiss; Chemie Bissendorf).

Administration of Nifedipine (Experiment 2)

Following a resting period of 2 hours, each volunteer received a capsule containing 10 mg nifedipine (Adalat 10-mg Kapseln; Bayer, Vienna, Austria). An infusion of vehicle was started, and 30 minutes thereafter, each volunteer was given IV bolus doses of 100 μg LHRH, 400 μg TRH, 100 μg hCRF, and 100 μg GHRH as described earlier.

Administration of Endothelin-1 (Experiment 3)

Following a resting period of 2 hours, each volunteer received an infusion of endothelin-1 (Peptides International, Louisville, KY). The preparation of endothelin-1 was previously shown to be chromatographically pure and nonpyrogenic. Endothelin-1 was dissolved in Haemaccel (Behring) and infused at a dose of 5 ng (2.0 pmol)/kg · min for 15 minutes, followed by 2.5 ng (1.0 pmol)/kg · min for 105 minutes. Thirty minutes after the beginning of this infusion, each volunteer was given bolus doses of 100 μg LHRH, 400 μg TRH, 100 μg hCRF, and 100 μg GHRH as described earlier.

Administration of Nifedipine and of Endothelin-1 (Experiment 4)

Following a resting period of 2 hours, each volunteer received a capsule containing 10 mg nifedipine. Simultaneously, an infusion of endothelin-1 was started. Thirty minutes thereafter, each volunteer was given IV bolus doses of 100 μg LHRH, 400 μg TRH, 100 μg hCRF, and 100 μg GHRH.

Two blood samples for determination of LH, follicle-stimulating hormone (FSH), PRL, thyrotropin-stimulating hormone (TSH), ACTH, and GH were drawn before infusion of either vehicle or endothelin-1. Further blood samples were obtained before (n = 2) the pituitary stimulation test and at 10-minute intervals thereafter.

Plasma concentrations of ACTH and TSH were determined radioimmunologically by means of commercially available kits

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(ACTH, Nichols, San Juan Capistrano, CA; TSH, Behring). GH was also determined radioimmunologically as described previously.[5] Commercially available ELISA methods (Syneliza, Freiburg, Germany) were used for determination of LH, FSH, and PRL. Interassay and intraassay coefficients of variation for all variables were less than 10%.

The data are presented as the mean ± SD. Duncan's multiple-range test[6] was used for statistical evaluation.

RESULTS

Blood Pressure

During the control experiment (experiment 1), mean blood pressure (BP_m) readings at the time of the pituitary stimulation test were identical (difference, 0 ± 3 mm Hg) to basal BP readings. Nifedipine alone (experiment 2) caused a decrease in mean BP (−7 ± 5 mm Hg). A slight increase in mean BP (2 ± 4 mm Hg) was observed during infusion of endothelin-1 alone (experiment 3), but not when administered together with oral nifedipine (experiment 4, −1 ± 8 mm Hg). The maximum increase in BP_m during infusion of endothelin-1 was 7.6 ± 3.0 mm Hg (endothelin-1 alone, experiment 3) and 4.3 ± 7.4 mm Hg (endothelin plus nifedipine, (experiment 4). No subjective or objective side effects were seen during any of the experiments.

ACTH

Plasma concentrations of ACTH were stimulated by CRF from a basal level of 28.8 ± 16.4 pg/mL to 58.8 ± 14.9 pg/mL during the control experiment (experiment 1). Whereas the CRF-induced increase in plasma ACTH concentrations was unchanged following pretreatment with oral nifedipine (experiment 2), it was enhanced during infusion of endothelin-1 (experiment 3; $P < .05$). This augmentation was reversed when nifedipine was administered at the beginning of infusion of endothelin-1 (experiment 4). The response of ACTH to CRF during this experiment was similar to control conditions (Table 1).

GH

The expected GHRH-induced increase in plasma concentrations of GH was seen during each of the four experiments (Table 2). Both nifedipine (experiment 2; $P < .01$) and endothelin-1 (experiment 3; $P < .01$) partially inhibited the effect of GHRH on GH secretion. An even more pronounced inhibition of GH secretion was seen following combined administration of nifedipine and endothelin (experiment 4).

PRL

The TRH-induced increase in plasma concentrations of PRL was unchanged by oral administration of 10 mg nifedipine (experiment 2). Endothelin-1 inhibited the TRH-stimulated increase in plasma PRL concentrations to a similar extent ($P < .01$) both with (experiment 3) and without (experiment 4) pretreatment with nifedipine (Table 3).

LH, FSH, and TSH

Plasma concentrations of LH, FSH, and TSH showed the expected increase following stimulation by LHRH and TRH, respectively. Neither nifedipine nor endothelin-1 alone nor the combination of these two drugs either further enhanced or suppressed the increase in plasma concentrations of any of these hormones (data not shown in detail).

Table 1. Plasma Concentrations of ACTH (pg/mL) in Healthy Volunteers (N = 6) Before and After Stimulation by CRF During a Control Experiment (experiment 1), Following 10 mg Oral Nifedipine (experiment 2), During an Infusion of Endothelin-1 (experiment 3), and During the Concomitant Administration of Nifedipine and Endothelin-1 (experiment 4)

Parameter	Exp 1	Exp 2	Exp 3	Exp 4
Basal 1	28.5 ± 17.6	24.8 ± 15.2	23.8 ± 14.0	24.7 ± 18.7
Basal 2	28.8 ± 16.4	25.8 ± 13.1	24.5 ± 12.1	24.3 ± 13.9
+10 min	47.0 ± 21.1	40.7 ± 16.6	43.2 ± 13.1	35.8 ± 13.4
+20 min	54.8 ± 19.0	43.7 ± 14.6	54.8 ± 20.5	40.7 ± 13.8
+30 min	54.8 ± 10.7	44.0 ± 14.6	60.0 ± 30.6	47.0 ± 12.1
+40 min	58.8 ± 14.9	48.5 ± 20.1	76.8 ± 35.8	50.3 ± 8.3
+50 min	56.0 ± 17.1	51.7 ± 17.8	67.8 ± 20.5	54.5 ± 13.6
+60 min	50.2 ± 16.9	46.5 ± 18.4	61.8 ± 16.7	50.8 ± 11.6
+70 min	42.7 ± 16.5	38.7 ± 15.2	57.8 ± 11.5	48.8 ± 11.8
+80 min	35.2 ± 17.8	34.3 ± 11.2	47.7 ± 16.8	40.1 ± 16.5
+90 min	30.8 ± 15.7	34.3 ± 14.0	37.5 ± 10.6	37.0 ± 15.8
Statistical evaluation				
v Exp 1	—	NS	<.05	NS
v Exp 2	NS	—	<.01	NS
v Exp 3	<.05	<.01	—	<.01
v Exp 4	NS	NS	<.01	—

NOTE. Basal concentrations (n = 2) were obtained before administration of endothelin and/or nifedipine (basal 1) and at the beginning of the pituitary stimulation test (basal 2).

Abbreviation: Exp, experiment.

Table 2. Plasma Concentrations of GH (ng/mL) in Healthy Volunteers (N = 6) Before and After Stimulation by GHRH During a Control Experiment (experiment 1), Following 10 mg Oral Nifedipine (experiment 2), During an Infusion of Endothelin-1 (experiment 3), and During the Concomitant Administration of Nifedipine and Endothelin-1 (experiment 4)

Parameter	Exp 1	Exp 2	Exp 3	Exp 4
Basal 1	0.7 ± 0.1	0.8 ± 0.3	1.1 ± 0.7	1.0 ± 0.8
Basal 2	0.7 ± 0.3	0.9 ± 0.2	1.1 ± 0.7	1.1 ± 0.9
+10 min	15.1 ± 10.4	11.2 ± 4.4	7.6 ± 3.0	9.0 ± 4.4
+20 min	23.8 ± 14.3	18.4 ± 6.6	14.4 ± 4.4	18.5 ± 8.7
+30 min	30.9 ± 18.3	24.3 ± 12.2	18.0 ± 4.8	20.3 ± 14.2
+40 min	33.7 ± 18.7	24.9 ± 11.8	19.6 ± 8.6	19.6 ± 12.0
+50 min	34.3 ± 20.0	22.5 ± 10.4	21.4 ± 9.7	21.5 ± 12.6
+60 min	31.8 ± 17.6	24.7 ± 11.0	26.0 ± 14.1	18.6 ± 11.2
+70 min	31.0 ± 19.1	22.3 ± 10.5	27.9 ± 15.8	18.0 ± 9.6
+80 min	26.2 ± 14.5	18.7 ± 11.2	33.8 ± 20.1	16.2 ± 8.4
+90 min	23.1 ± 13.3	18.6 ± 11.8	38.0 ± 23.4	15.0 ± 8.1
Statistical evaluation				
v Exp 1	—	<.01	<.01	<.05
v Exp 2	<.01	—	NS	NS
v Exp 3	<.01	NS	—	<.01
v Exp 4	<.05	NS	<.01	—

NOTE. Basal concentrations (n = 2) were obtained before administration of endothelin and/or nifedipine (basal 1) and at the beginning of the pituitary stimulation test (basal 2).

Table 3. Plasma Concentrations of PRL (ng/mL) in Healthy Volunteers (N = 6) Before and After Stimulation by TRH During a Control Experiment (experiment 1), Following 10 mg Oral Nifedipine (experiment 2), During an Infusion of Endothelin-1 (experiment 3), and During the Concomitant Administration of Nifedipine and Endothelin-1 (experiment 4)

Parameter	Exp 1	Exp 2	Exp 3	Exp 4
Basal 1	4.7 ± 1.5	5.2 ± 1.7	4.9 ± 2.5	4.6 ± 1.6
Basal 2	4.4 ± 1.7	5.4 ± 1.7	4.0 ± 1.1	4.2 ± 1.1
+10 min	24.9 ± 6.5	24.4 ± 4.5	19.7 ± 3.8	22.8 ± 2.7
+20 min	24.8 ± 7.1	25.3 ± 4.5	20.5 ± 4.0	21.8 ± 2.7
+30 min	20.9 ± 4.7	21.6 ± 4.6	18.5 ± 3.5	19.5 ± 2.8
+40 min	18.8 ± 4.7	18.7 ± 4.4	15.7 ± 3.0	16.9 ± 2.4
+50 min	16.5 ± 4.2	16.4 ± 3.6	13.8 ± 2.7	14.1 ± 2.2
+60 min	13.7 ± 3.2	13.5 ± 2.7	11.7 ± 2.0	11.5 ± 2.4
+70 min	12.2 ± 2.9	12.2 ± 3.0	10.4 ± 1.6	10.6 ± 2.2
+80 min	10.8 ± 2.3	10.8 ± 2.4	9.4 ± 2.0	9.4 ± 2.0
+90 min	9.7 ± 1.9	9.5 ± 2.4	8.7 ± 1.7	8.6 ± 2.0
Statistical evaluation				
v Exp 1	—	NS	<.01	<.01
v Exp 2	NS	—	<.01	<.01
v Exp 3	<.01	<.01	—	NS
v Exp 4	<.01	<.01	NS	—

NOTE. Basal concentrations (n = 2) were obtained before administration of endothelin and/or nifedipine (basal 1) and at the beginning of the pituitary stimulation test (basal 2).

DISCUSSION

Although pituitary cell populations show some variability with regard to sensitivity to endothelin-1 and to other vasoactive peptides,[7] endothelin-1 has been shown in vitro to enhance gonadotropin secretion[8] and to inhibit the secretion of PRL,[9] the latter effect being reversible by long-term exposure to dopamine.[10] Endothelin-1 has been shown to increase basal ACTH release and to suppress the release of ACTH induced by CRF in vitro.[11] In healthy men, pharmacological doses of endothelin-1 suppress TRH (GHRH)-stimulated plasma concentrations of PRL and GH, but augment the CRF-induced release of ACTH.[2] These results, as well as the presence of immunoreactivity, messenger RNA, and binding sites for endothelin in human pituitary tissue,[12] indicate that some members of the endothelin family, particularly endothelin-1 and endothelin-3, may be involved in pituitary hormone secretion. Indeed, besides their vasopressor action[13] endothelins apparently also exert some influence in various endocrine systems such as the adrenals, gonads, thyroid, parathyroid, and glucose metabolism.[14-17]

Ever since the first description of endothelin-1, it has been suggested that the peptide's effect, at least on vascular smooth muscle, is largely mediated by modulation of dihydropyridine-sensitive calcium channels.[18] The release of intracellular calcium is also involved in the effect of endothelin-1 in brain and adenohypophysis tissues.[1] The metabolic activation (ie, oxidation of glucose) that is increased by IV infusion of endothelin-1 in the rat pituitary is antagonized by the blockade of dihydropyridine-sensitive calcium channels,[4] indicating that endothelin stimulates local energy metabolism via L-type calcium channels. Since these findings apparently identified dihydropyridine-sensitive calcium channels as an important pathway in the metabolic response of pars intermedia and distalis to central endothelin, it appeared of interest to evaluate whether the previously observed effects of IV endothelin-1 on the secretion of pituitary hormones in vivo would be reversible by preadministration of a calcium channel blocker.

However, this model is complex, since calcium antagonists per se may exert an effect on pituitary hormone secretion. In vitro, nifedipine inhibits the stimulated release of LH and PRL.[19] Evidence in vivo is contradictory. In healthy subjects, nifedipine suppresses TRH-induced release of TSH but not of PRL.[20] Verapamil has been shown to inhibit the release of TSH, LH, and FSH but not of PRL stimulated by TRH and LHRH, respectively,[21] but no effect on any of these hormones was seen during a subsequent investigation using nifedipine.[22] These differences have led to the suggestion that calcium antagonists could also influence pituitary function by mechanisms other than antagonism of calcium influx.[22] In accordance with the data reported by Struthers et al,[22] nifedipine failed to influence the secretion of LH, FSH, PRL, and TSH in our healthy volunteers. However, there was a decrease in GHRH-stimulated GH secretion. The unchanged CRF-induced increase of ACTH, which is at variance with data reported by others,[23] could be due to the smaller (10 *v* 20 mg) dose of nifedipine and the different stimulus (CRF *v* AVP) used in our investigation.

With regard to the effects of endothelin on anterior pituitary function in vivo, the results of the present investigation confirm our previously reported[2] data. In healthy men, IV endothelin did not influence TRH- and LHRH-stimulated secretion of TSH, LH, and FSH, whereas the release of GH and PRL was diminished and CRF-induced secretion of ACTH was enhanced. Pretreatment with nifedipine reversed the latter effect but augmented the inhibitory effect of endothelin-1 on the secretion of GH and PRL.

Identical infusion rates of endothelin-1 result in higher plasma concentrations of endothelin-1 in healthy men pretreated with nifedipine, possibly due to a decreased clearance rate of endothelin-1.[24] Thus, although local pituitary concentrations of endothelin-1 in man during exogenous administration of the peptide are unknown, the interaction of nifedipine and endothelin with regard to the secretion of GH and PRL could be the result of higher prevailing local concentrations of endothelin-1. However, with regard to the simultaneous reversal by nifedipine of the endothelin-1–dependent changes in CRF-stimulated ACTH secretion, this is not a likely explanation. Following the same line of argument, the discrepant influence of nifedipine on endothelin-dependent changes in ACTH versus PRL and GH does not permit the conclusion that the effect of endothelin-1 on pituitary hormone secretion in vivo is exclusively mediated by dihydropyridine-sensitive calcium channels.

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REFERENCES

1. Stojilkovic SS, Balla T, Fukuda S, et al: Endothelin ET_A receptors mediate the signaling and secretory actions of endothelins in pituitary gonadotrophs. Endocrinology 130:465-474, 1992
2. Vierhapper H, Hollenstein U, Roden M, et al: Effect of endothelin-1 in man—Impact on basal and stimulated concentrations of luteinizing hormone, follicle-stimulating hormone, thyrotropin, growth hormone, corticotropin, and prolactin. Metabolism 42:902-906, 1993
3. Kiowski W, Lüscher TF, Linder L, et al: Endothelin-1 induced vasoconstriction in man: Reversal by calcium channel blockade but not by nitrovasodilators or endothelium-derived relaxing factor. Circulation 83:469-475, 1991
4. Gross PM, Wainman DS, Espinosa FJ: Differentiated metabolic stimulation of rat pituitary lobes by peripheral and central endothelin-1. Endocrinology 129:1110-1112, 1991
5. Waldhäusl W, Rath F: Development of antibodies to human growth hormone (hGH) in children of short stature with hGH. Acta Endocrinol (Copenh) 68:345-354, 1971
6. SAS: SAS User's Guide: Statistics. Cary, NC, SAS Institute, 1982
7. Stojilkovic SS, Iida T, Merelli F, et al: Calcium signaling and secretory responses in endothelin-stimulated anterior pituitary cells. Mol Pharmacol 39:762-770, 1991
8. Stojilkovic SS, Merelli F, Iida T, et al: Endothelin stimulation of cytosolic calcium and gonadotropin secretion in anterior pituitary cells. Science 248:1663-1666, 1990
9. Samson WK, Skala KD, Alexander BD, et al: Pituitary site of action of endothelin: Selective inhibition of prolactin release in vitro. Biochem Biophys Res Commun 169:737-743, 1990
10. Kanyicska B, Livingstone JD, Freeman ME: Long term exposure to dopamine reverses the inhibitory effect of endothelin-1 on prolactin secretion. Endocrinology 136:990-994, 1995
11. Calogero AE, Raiti F, Nicolosi G, et al: Effects of endothelin-1 and endothelin-3 on rat hypothalamic corticotrophin-releasing hormone and pituitary ACTH release in vitro. J Endocrinol 140:419-424, 1994
12. Takahashi K, Ghatei MA, Jones PM, et al: Endothelin in human brain and pituitary gland: Presence of immunoreactive endothelin, endothelin messenger ribonucleic acid, and endothelin receptors. J Clin Endocrinol Metab 72:693-699, 1991
13. Vierhapper H, Wagner O, Nowotny P, et al: Effect of endothelin-1 in man. Circulation 81:1415-1418, 1990
14. Vierhapper H, Nowotny P, Waldhäusl W: Effect of endothelin-1 in man: Impact on basal and adrenocorticotropin-stimulated concentrations of aldosterone. J Clin Endocrinol Metab 80:948-951, 1995
15. Vierhapper H: Endocrine effects of endothelin-1. Endothelium 3:113-120, 1995
16. Roden M, Vierhapper H, Liener K, et al: Endothelin-1–stimulated glucose production in vitro in the isolated perfused rat liver. Metabolism 41:290-295, 1992
17. Roden M, Prskavec M, Fürnsinn C, et al: Evidence for phosphoramidon-sensitive cleavage of big endothelin-1 involved in endothelin-stimulated hepatic glucose production. Regul Pept 51:207-213, 1994
18. Yanagisawa M, Kurihara H, Kimura S, et al: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332:411-415, 1988
19. Takahara J, Yunoki S, Yamane Y, et al: Effects of nifedipine on prolactin, growth hormone, and luteinizing hormone release by rat anterior pituitary in vitro. Proc Soc Exp Biol Med 162:31-33, 1979
20. Brabant G, Prank H, Hoang-Vu C, et al: Hypothylamic regulation of pulsatile thyrotropin secretion. J Clin Endocrinol Metab 72:145-150, 1991
21. Barbarino A, de Marinis L: Calcium antagonists and hormone release. II. Effects of verapamil on basal, gonadotropin-releasing hormone and thyrotropin-releasing hormone induced pituitary hormone release in normal subjects. J Clin Endocrinol Metab 51:749-753, 1980
22. Struthers AD, Millar JA, Beastal GH, et al: Calcium antagonists and hormone release: Effect of nifedipine on luteinizing hormone–releasing hormone and thyrotropin-releasing hormone–induced pituitary hormone release. J Clin Endocrinol Metab 56:401-404, 1983
23. Jackson RV, Jackson AJ, Grice JE, et al: Nifedipine blocks ACTH and cortisol release in man. Clin Exp Pharmacol Physiol 16:257-261, 1989
24. Vierhapper H, Wagner OF, Nowotny P, et al: Effect of endothelin-1 in man: Pretreatment with nifedipine, with indomethacin and with cyclosporine A. Eur J Clin Invest 22:55-59, 1992