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RELEASE OF β-ENDORPHIN-IMMUNOREACTIVITY FROM RAT PITUITARY AND HYPOTHALAMUS IN VITRO: EFFECTS OF ISOPROTERENOL, DOPAMINE, CORTICOTROPIN-RELEASING FACTOR AND ARGININE^8-VASOPRESSIN

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SUMMARY The release of β-endorphin-immunoreactivity (βE-IR) from rat pituitary anterior lobe (AL) quarters, neurointermediate lobes (NILs), and hypothalamic fragments was investigated in vitro. The β-adrenoceptor agonist isoproterenol (ISO) and the hypothalamic neurohormone corticotropin-releasing factor (CRF) concentration-dependently stimulated the release of βE-IR from superfused AL quarters and NILs, but not from incubated hypothalamic fragments. Dopamine (DA) inhibited the release of βE-IR from NILs and hypothalamic tissue in a concentration-dependent manner, whereas it did not affect the release from AL quarters. Arginine^8-vasopressin (AVP) stimulated the release of βE-IR from AL quarters and hypothalamic fragments, but did not affect the release from NILs. The data indicate that the release of βE-IR from cells in the pituitary lobes and in the hypothalamus is differentially regulated, but that common principles are involved. In particular, the results provide first direct evidence for an action of vasopressin as a stimulator of the release of POMC-derived peptides in the hypothalamus.

β-LPH, β-endorphin (βE) and related peptides are formed by enzymatic processing of the multifactorial precursor molecule pro-opiomelanocortin (POMC) (1). POMC is produced in a variety of tissues including the corticotroph cells of the anterior lobe (AL), the melanotroph cells of the intermediate lobe of the pituitary gland, and in certain neuronal cells in the brain (2-4). The major site of POMC production in the brain is found in the arcuate nucleus region of the hypothalamus (4,5).

The regulation of the secretory activity of the pituitary POMC cells is well investigated. The secretion of POMC-derived peptides from the AL is stimulated by corticotropin-releasing factor (CRF) and arginine^8-vasopressin (AVP) both in vivo and in vitro (6,7), while their secretion from the neurointermediate lobe (NIL) of the pituitary is stimulated by the

Abbreviations: βE, β-endorphin; βE-IR, β-endorphin-immunoreactivity; POMC, pro-opiomelanocortin; ISO, isoproterenol; DA, dopamine; CRF, corticotropin-releasing factor; AVP, arginine^8-vasopressin; AL, anterior lobe; NIL, neurointermediate lobe; CSF, cerebrospinal fluid; RIA, radioimmunoassay.

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β-adrenoceptor agonist isoproterenol (ISO) and inhibited by dopamine (DA) (8).

Although abundant evidence is available that POMC-derived peptides affect a variety of brain functions and act as neuromodulators or neurohormones in the brain (9,10), relatively little is known on the regulation of the activity of POMC producing neurons in the brain. We have recently shown that intracerebroventricular (i.c.v.) administration of AVP in rats induces an increased concentration of βE-IR in the cerebrospinal fluid (CSF) without influencing pituitary βE-IR secretion (11, Barna et al., submitted), whereas ISO and CRF were ineffective in this respect. This suggests, that vasopressin may act as a releaser of βE and other POMC-derived peptides in the brain. Therefore, it was of interest to study the effects of ISO, DA, CRF and AVP on the release of βE-IR from hypothalamic tissue in vitro, in comparison with their effects on the release of βE-IR from superfused pituitary lobes.

MATERIALS AND METHODS

Animals and tissue collection

Male albino Wistar rats bred from own stock (Cpb:WU) weighing 200-240 g were used for the experiments. The animals were maintained in a temperature- (23 + 1 °C) and light- (10 h light - 14 h dark regimen) controlled environment, and received standard diet and tap water ad libitum.

Rats were decapitated between 9.30 and 10.30 a.m. The brain was removed from the skull and the hypothalamus (wet weight 12-17 mg) was dissected within 45 sec of decapitation by transverse cuts along the rostral edges of the mammillary bodies and optic chiasm, sagittal cuts following the lateral hypothalamic fissures, and a horizontal cut at the level of the top of the third ventricle. The pituitary was taken out of the skull and the NIL was carefully separated from the AL. Each AL was subsequently cut into quarters. Immediately following dissection, the tissues were placed in vials containing ice-cold carbogenated medium.

Superfusion of pituitary tissue

AL quarters or NILs were transferred to a superfusion apparatus (2 lobes/chamber), and superfused with carbogenated Krebs-Ringer bicarbonate buffer (118 mM NaCl, 4.85 mM KCl, 1.15 mM KH_2PO_4, 1.15 mM $MgSO_4.7H_2O$, 25 mM $NaHCO_3$, 1.25 mM $CaCl_2.2H_2O$) containing 5 $g.l^{-1}$ bovine serum albumin (BSA, Sigma), 0.1 mM ascorbic acid and 11.1 mM glucose (medium), and kept at 37 °C. In all experiments, the lobes were first superfused for 90 min to obtain steady basal rates of βE-IR release (equilibration period). During the remainder of the experiment, superfusate fractions (10 min; 2 ml) were collected. Following the equilibration period, superfusion was continued for 60 min with medium. Subsequently, the lobes were exposed for 30 min to medium containing ISO (isoprenalini sulfas, OPG, The Netherlands), DA (dopamine-HCl, Sigma, USA), human/rat CRF (Peninsula Lab. Inc.) or AVP (Organon International B.V., Oss, The Netherlands). Thereafter, superfusion was continued for another 70 min with initial medium. Each drug was tested in a separate experiment, including three concentrations of the drug (n=4-6).

In vitro incubation of hypothalamic fragments

Hypothalami were bidirectionally sliced with a McIlwain tissue chopper (300 μm) and tissue fragments equivalent to 5 hypothalami were placed in an

incubation chamber. The fragments were preincubated at 37 °C for 15 min in 1 ml medium containing 0.1 $g.l^{-1}$ BSA, under an atmosphere of 95% O_2 and 5% CO_2. After preincubation, the medium was aspirated and replaced by 0.5 ml fresh medium. Subsequently, the medium was replaced by the same volume of fresh medium every 15 min throughout the experiment. After 5 subsequent 15 min incubation periods, the medium was changed for medium containing 45 mM K^+, ISO, DA, CRF or AVP (10^{-6}M, 10^{-7}M, 10^{-8}M). After the stimulus (two 15 min periods), the media were replaced by initial medium and incubation was continued for three 15 min periods. Per experiment 9 incubations were performed, and incubations of fragments in medium containing one concentration of a drug (n=4-5) and control incubations of fragments in medium without additions (n=4-5) were run in parallel.

β-endorphin radioimmunoassay

All samples were collected in ice-chilled polystyrene tubes containing 200 KIU Trasylol^R (Aprotonine, Bayer, Leverkusen). A rabbit antiserum was used, raised against synthetic camel β-endorphin-(1-31) (β_cE-(1-31); antiserum X7). The antiserum was directed against the C-terminal part of this peptide. Synthetic β_cE-(1-31) was used as standard and [^{125}I]β_cE-(1-31) as tracer. The sensitivity of the assay was 2-5 pg per tube (at 10% displacement of tracer). The following cross-reactivities (expressed as % on mass basis) were obtained: human β-LPH, 83% ; Acβ_cE-(1-31), 85% ; β_cE-(1-27), 2% and Acβ_cE-(1-27), 0.6%. Cross-reactivity with βE-(1-26), Acβ_cE-(1-26), γE (βE-(1-17)), αE (βE-(1-16)), α-MSH and Met-Enk was < 0.1%. Medium and medium containing 10^{-6}M ISO, DA, CRF or AVP did not interfere in the radioimmunoassay (RIA) system.

RIA's were performed in essence as described earlier (12). All assays were performed in triplicate. Sample dilution curves parallelled the standard curves.

Calculations

The total βE-IR released from superfused AL quarters and NILs during a stimulus is expressed as percentage of the calculated basal release. The basal release was computed from the area under the line linking the means of the βE-IR content of the two superfusate fractions immediately preceding, and of the first two fractions following stimulation where stabilization of the release had again occurred.

The total βE-IR released from hypothalamic fragments during a stimulus is expressed as percentage of the total amount of βE-IR released in fractions 6 and 7 obtained from parallel incubations of tissue fragments with control medium (cf. Fig. 1).

Data are presented as mean ± S.E. Statistical evaluation was performed per experiment using Student's t-test.

RESULTS

βE-IR release from pituitary lobes

The spontaneous release of βE-IR from superfused rat AL quarters and NILs was high during the initial phase of the equilibrium period (800-1000 pg/AL/10 min and 1000-1500 pg/NIL/10 min). Then it rapidly declined and reached a rather stable basal level (250-400 pg/AL/10 min and 300-500 pg/NIL/10 min) after approximately 90 min, which was maintained throughout the remainder of the experiment (data not shown).

AL quarters and NILs were superfused for 30 min with medium containing 10^{-6}-10^{-8}M ISO or DA, or 10^{-7}-10^{-9}M CRF or AVP. When effective, the drugs induced a rapid, sustained effect on the release of βE-IR and removal of the

Table I. Effects of various drugs on the release of βE-IR from rat anterior and neurointermediate pituitary and from hypothalamus in vitro

drug	conc.(M)	AL %	NIL %	hypothalamus %
isoproterenol	10^{-6}	131.8 ± 7.6**	245.2 ± 10.8***	91.3 ± 9.7
	10^{-7}	117.3 ± 4.7*	173.6 ± 8.9***	128.5 ± 11.2
	10^{-8}	111.3 ± 3.8	141.2 ± 3.2***	88.3 ± 14.5
dopamine	10^{-6}	95.0 ± 5.9	48.9 ± 3.7***	71.1 ± 6.9**
	10^{-7}	99.3 ± 6.0	65.7 ± 4.4***	63.9 ± 2.9**
	10^{-8}	97.4 ± 5.7	84.9 ± 2.7*	86.1 ± 7.4
CRF	10^{-6}	N.D.	N.D.	97.5 ± 8.6
	10^{-7}	768.5 ± 67.6***	151.5 ± 2.1***	109.3 ± 12.5
	10^{-8}	564.3 ± 37.7***	131.8 ± 6.1**	88.1 ± 8.6
	10^{-9}	273.0 ± 22.8***	109.7 ± 4.7	N.D.
AVP	10^{-6}	N.D.	N.D.	142.9 ± 12.9*
	10^{-7}	795.3 ± 52.0***	95.7 ± 6.9	185.3 ± 28.4*
	10^{-8}	504.0 ± 21.5***	104.3 ± 4.7	107.1 ± 24.3
	10^{-9}	281.1 ± 28.3***	112.8 ± 6.9	N.D.

Concentration of βE-IR in superfusates and incubation media after exposure of AL quarters (n=4-6), NILs (n=4-6), and hypothalamic fragments (n=4-5) to different concentrations of ISO, DA, CRF or AVP. Data are expressed as percentage of the computed basal release (AL, NIL) or of the amount of βE-IR released during parallel incubations of tissue fragments with control medium (hypothalamus), and are given as mean ± S.E. Significant differences vs control: * P<0.05, ** P<0.01, *** p<0.001 (Student's t-test). N.D.: not determined.

drugs resulted in a rapid return of release to basal levels (not shown). The results are presented in Table I. Superfusion of AL quarters with ISO stimulated the release of βE-IR in a concentration-dependent manner. The stimulation reached statistical significance at ISO concentrations of 10^{-6}M and 10^{-7}M. The response of NILs to ISO was considerably larger, and statistically significant at all three concentrations tested. Superfusion with DA did not alter the βE-IR release from AL quarters, whereas it induced a concentration-dependent inhibition of the βE-IR release from NILs. CRF elicited a concentration-dependent increase in the release of βE-IR from both AL quarters and NILs. The effect on NILs however, was considerably smaller than that on AL quarters, and reached statistical significance only at CRF concentrations of 10^{-7}M and 10^{-8}M. AVP induced a concentration-dependent increase in the release of βE-IR from AL quarters which was comparable to that found with CRF. AVP did not affect the release of βE-IR from NILs in the concentrations tested.

βE-IR release from hypothalamic fragments

In vitro incubation of hypothalamic fragments resulted in a spontaneous release of βE-IR into the medium as shown in figure 1A. The

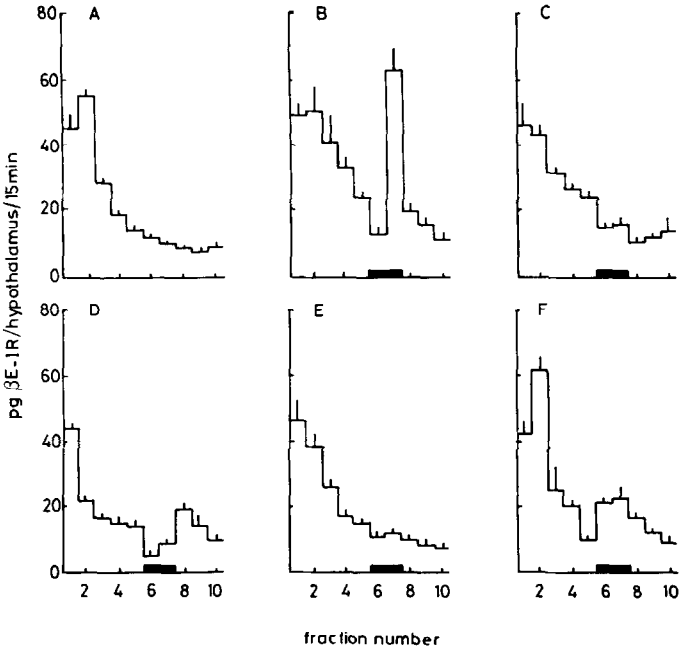

Fig 1. Spontaneous and stimulus-induced release of βE-IR from hypothalamic fragments in vitro. Fragments from 5 hypothalami were incubated in medium alone (panel A), or were incubated in medium and given 30 min pulses of medium containing 45 mM K^+ (panel B), 10^{-7}M ISO (panel C), DA (panel D), CRF (panel E) or AVP (panel F). Data are expressed as pg βE-IR released per hypothalamus per 15 minutes and represent the mean ± S.E. of 4-6 observations.

initial rate of release of βE-IR was high and varied between 30-60 pg/hypothalamus/15 min. It rapidly declined during the first hour of the incubation period and then continued at a rather stable level of 8-15 pg/hypothalamus/15 min for at least 90 min. Incubation of tissue fragments in medium containing 45 mM K^+ resulted in a significant increase (+104%; $p<0.001$) in the concentration of βE-IR in the medium as compared to control (Fig. 1A and 1B). Subsequent incubation with initial medium resulted in a return of the release of βE-IR to basal levels.

In order to determine whether factors that affected the release of βE-IR from the pituitary lobes influence the release of βE-IR from hypothalamic tissue, we incubated hypothalamic fragments with 10^{-6}-10^{-8}M ISO, DA, CRF or AVP (Table I). In Figure 1C-F typical patterns of the release of βE-IR from hypothalamic fragments in response to respectively 10^{-7}M ISO, DA, CRF and AVP are presented. Incubation of tissue fragments for 30 min with medium containing ISO did not alter the βE-IR release. DA inhibited the release of βE-IR in a concentration-dependent manner. The inhibition reached statistical significance at 10^{-6}M and 10^{-7}M. CRF did not affect the release of βE-IR from the tissue fragments. There was a concentration-dependent increase in the release of βE-IR from hypothalamic tissue following

incubation with AVP. Incubation of hypothalamic tissue with medium containing 10^{-7}M AVP resulted in a larger response (+85%) than incubation of tissue fragments with 10^{-6}M AVP (+43%).

DISCUSSION

This study was carried out to investigate the effects of various drugs on the release of βE-IR from the hypothalamus in vitro, as compared with the anterior and neurointermediate lobe of the pituitary. The viability of the hypothalamic fragments was validated by examining the secretory response to a depolarizing concentration of potassium. The results showed that in vitro incubation of hypothalamic fragments can be used as a model to investigate hypothalamic βE-IR release.

The present results demonstrating that ISO stimulates the release of βE-IR from AL quarters in vitro are consistent with findings that ISO stimulates the release of POMC-derived peptides from the AL in vivo (13,14), and from cultured pituitary corticotrophs in vitro (15). Other investigators however, found no influence of ISO on the secretion of βE-IR from dispersed anterior pituitary cells (8). This latter finding may be explained by the fact that enzymatically dissociated pituitary corticotrophs display a diminished response to secretagogues (16). Thus, β-adrenergic mechanisms in the anterior pituitary may be involved in the release of βE-IR. The observation that the release of βE-IR from NILs is stimulated by ISO is in agreement with reports that the secretion of peptides from the intermediate lobe in vitro and in vivo is stimulated by activation of β-adrenoceptors (17,18). Unlike the NIL, however, ISO did not affect βE-IR release from hypothalamic fragments. This finding confirms that of Vermes et al. (19), and is in line with our previous in vivo data showing that i.c.v. administration of ISO to rats does not alter the concentration of βE-IR in the CSF (Barna et al., submitted).

In accordance with literature data (8), DA inhibited the release of βE-IR from NILs in vitro while it had no effect on AL quarters. In addition, DA inhibited the release of βE-IR from hypothalamic fragments, which underscores earlier findings that DA inhibits both spontaneous and K^+-induced βE and β-LPH release from hypothalamic slices through activation of D_2-receptors (19). The inhibition by DA of hypothalamic βE-IR release resembles the situation in the NIL where peptide release can also be reduced by a D_2-receptor mediated mechanism (20). Thus, DA seems to be involved in the regulation of the activity of neurointermediate pituitary and hypothalamic βE producing cells. This is supported by observations that chronic treatment of rats with the DA receptor agonist bromocriptine resulted in a decreased concentration of βE-IR in the hypothalamus and the

NIL, whereas treatment with the DA receptor antagonist haloperidol increased these levels (21,22, Sweep et al., submitted).

The CRF-elicited increase in the release of βE-IR from AL quarters and NILs is consistent with studies showing that CRF is a potent modulator of βE-IR release from the AL, while it is a less potent secretagogue for the NIL (6,23). We did not find an effect of CRF on the basal release of βE-IR from hypothalamic fragments. The present in vitro data therefore extend earlier studies, showing that i.c.v. administration of CRF in rats does not alter the concentration of βE-IR in the CSF (Barna et al., submitted). Our findings are at variance, however, with data from Nikolarakis et al. (24). These authors showed that CRF concentration-dependently stimulates the release of βE-IR from superfused hypothalamic slices. Differences in experimental procedures (strain of rats, tissue dissection and preparation, superfusion vs incubation of tissue, composition of media, antisera) may underlie this discrepancy.

The present results demonstrate that AVP stimulates the release of βE-IR from hypothalamic tissue in vitro, and suggest that AVP may regulate the activity of hypothalamic POMC neurons. They are in accord with previous data showing that the concentration of βE-IR in the CSF is increased following i.c.v. administration of AVP (11, Barna et al., submitted). Specific binding of the pressor antagonist $d(CH_2)_5Tyr(Me)VP$ to slices of rat brain has been shown, suggesting the presence of vasopressin receptors, in particular in the arcuate nucleus region (25). Thus, the arcuate nucleus of the hypothalamus might be an anatomical target for AVP to act on POMC neurons. In conclusion, the data indicate that AVP is a releaser of βE-IR from the hypothalamus, and suggest that vasopressin functions as a releasing factor for POMC-derived peptides in the hypothalamus, like it does in the anterior pituitary.

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