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Plasma concentration and vascular effect of β-endorphin in spontaneously hypertensive and Wistar Kyoto rats*

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Summary. In order to find out whether β-endorphin (β-E) is involved in the development of hypertension, we performed two series of experiments. Firstly, spontaneously hypertensive rats (SHR) and their normotensive Wistar Kyoto controls (WKY) were submitted to ether stress. Plasma concentrations of β-endorphin-like immunoreactivity (β-EI), adrenocorticotropin (ACTH) and α-melanotropin (α-MSH) were measured by radioimmunoassay. The basal concentration of β-EI was similar in WKY and SHR, whereas WKY had higher levels of ACTH and lower levels of α-MSH than SHR. In both strains acute stress enhanced the plasma concentration of β-EI to the same extent and with a similar time-course. The increase of plasma β-EI coincided with a rise in ACTH but not α-MSH. Gel chromatography of β-EI revealed that plasma extracts contain similar amounts of β-lipotropin- (β-LPH) and β-E-sized immunoreactive components, and that acute stress elevated both forms of β-EI. Secondly, isolated tail arteries of SHR and WKY were perfused and field stimulated with two pulses at 1 Hz. β-E depressed stimulation-evoked vasconstriction with the same potency in both strains. Thus, basal and stress-induced levels of β-EI did not differ in SHR and WKY. Moreover, in the tail artery of both strains the sensitivity of presynaptic opioid receptors towards β-E was almost identical. If the β-E sensitivity of these receptors in other arteries of WKY and SHR is also similar, a major role of the circulating peptide in the development of hypertension is rather unlikely.

Key words: Presynaptik opioid receptors – β-Endorphin – ACTH – α-MSH – Rat tail artery – Spontaneously hypertensive rats – Stress

Introduction

It has been suggested that opioid peptides play a key role in the central regulation of blood pressure (see Holaday 1983; McQueen 1983). However, these peptides may also produce hypotension by activating opioid receptors situated at the terminals of postganglionic sympathetic nerves innervating blood vessels (see Illes and Pfeiffer 1985). Receptor-activation may be followed by a decrease in action potential-induced transmitter release and consequently in vasoconstriction. Presynaptic opioid receptors in peripheral organs are not homogenous (Hughes 1981). In different arteries of rabbits, for example, only δ- and/or κ-, but no μ-receptors have been described (Illes et al. 1985; von Kügelgen et al. 1985; Illes et al. 1986b). In addition we have recently identified in the rat tail artery a presynaptic opioid receptor, which belongs to a β-endorphin-sensitive ε-type, and suggested that it may be a target of both locally released and blood-borne opioid peptides (Illes et al. 1987).

The involvement of centrally acting β-E in the antihypertensive action of clonidine in spontaneously hypertensive rats (SHR), in contrast to their normotensive Wistar Kyoto controls (WKY), has been recently proposed (Kunos et al. 1981; Ramirez-Gonzalez et al. 1983). The reduction of blood pressure induced by clonidine or α-methyldopa was counteracted by naloxone (Farsang et al. 1980) or by specific antibodies raised against human β-E (Ramirez-Gonzalez et al. 1983). It has also been reported that clonidine releases β-E into the systemic circulation in rats (Pettibone and Mueller 1981), as well as in humans (Farsang et al. 1984). Although elevated levels of β-endorphine-like immunoreactivity (β-EI) were found in the neurointermediate lobe of the pituitary of SHR, the plasma concentration of β-EI was lower in SHR than in WKY (Hutchinson et al. 1981).

Taken together, these results suggest that in addition to the central effect of β-E, peripheral mechanisms may be also involved in the development of hypertension. In order to prove this hypothesis two series of experiments were performed. Firstly, we determined whether if the basal and stress-induced release of plasma β-EI is different in SHR and WKY. Secondly, we investigated whether the potency of β-E to reduce nerve stimulation-evoked vasoconstriction differs in tail arteries of SHR and WKY.

Methods

Animals. Male SHR and WKY (originating from the Okamoto strain) were bred in our laboratory. They were housed in an air conditioned room under controlled conditions of light (12 h light from 7.00 a.m. to 7.00 p.m.) and temperature (22° C) and received food and water ad libitum. Twelve week old animals were used in this study. Their systolic blood pressure was measured by the tail cuff method; it was higher than 170 mmHg in SHR and below 125 mmHg in WKY.

Ether stress. One day before the experiments, the animals were separated and caged in groups of 2. Basal plasma levels

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of hormones were measured in rats decapitated immediately after removal from their cage. Acute ether stress was induced by exposing the animals to an atmosphere saturated with ether vapour for 1 min. The animals were decapitated at the indicated times. Trunk blood was collected in pre-cooled siliconized centrifuge tubes containing EDTA (1 mg · ml^{-1}) and aprotinin (300 UIK · ml^{-1}). Plasma was separated by centrifugation at 4°C and plasma samples were stored at −20°C until assayed.

Radioimmunoassay. Levels of β-EI in plasma were estimated by radioimmunoassay without prior extraction as previously described (Anhut et al. 1981). An anti-serum raised against human β-E was employed at a final dilution of 1:60,000 (for buffer) and 1:30,000 (for plasma). The cross-reactivity of human β-lipotropin (β-LPH) with the anti-β-E antiserum was about 50% on a molar basis; endogenous opioids such as enkephalins or dynorphin, α-MSH and ACTH did not cross-react (Knepel et al. 1983). All compounds used in the radioimmunoassay were dissolved or diluted further in 50 mM Tris/HCl-buffer, pH 8.0 containing 0.2% bovine serum albumin. When standard curves were determined, the incubation mixture contained human β-E in the range of 2−600 fmol; for measurements in plasma samples the appropriate volume of plasma, freed of endogenous β-E by adsorption onto charcoal, was present. All β-E values are expressed as human β-E equivalents. Plasma adrenocorticotropin (ACTH) was measured using a highly selective ACTH radioimmunoassay kit. Plasma α-melanotropin (α-MSH) was assayed using a radioimmunoassay method as previously described; the antiserum used is highly specific and directed against the C-terminal part of the peptide (Schmitt et al. 1979).

Plasma extraction and gel filtration. Pooled plasma samples from basal and ether-stressed rats were immediately extracted using silicic acid. The eluates were dried. The dry residues were reconstituted with 0.7 ml buffer and centrifuged at 4°C for 15 min; then the supernatant was subjected to gel filtration on a Sephadex G-50 superfine column (1.2 × 80 cm). The column was eluted (15 ml · h^{-1}, 1.25 ml fractions) at 4°C with the same buffer. Aliquots were analyzed by radioimmunoassay for β-EI as described above. Dextran blue (void volume), human β-E, ribonuclease (13.7 kDa; molecular size marker) and diazotized sulfanil acid (total volume) were used to characterize the column.

Rat tail artery. The preparation and experimental procedure was similar to that previously described (Illes et al. 1987). Briefly, proximal segments of 1.5−2.5 cm of tail arteries of SHR and WKY were dissected out and cannulated at both ends. The tissues were suspended vertically in an organ bath containing 4 ml of medium and perfused at 2.1 ml · min^{-1}, with the same medium which consisted of (mmol · l^{-1}) NaCl, 118; KCl, 4.8; $CaCl_2$, 2.5; $KHPO_4$, 0.9; $NaHCO_3$, 25 and glucose 11. It was saturated with 95% O_2 and 5% CO_2 and maintained at 37°C. The arteries were allowed to equilibrate for 1 h before electrical stimulation was started. The periarterial sympathetic nerves were stimulated via platinum electrodes located at the top and the bottom of the organ bath. Two field pulses of 0.3 ms pulse duration and 200 mA current strength were delivered at 1 Hz every 2 min with a Hugo Sachs Stimulator I. Vasoconstrictor responses were measured as increased in perfusion pressure with a

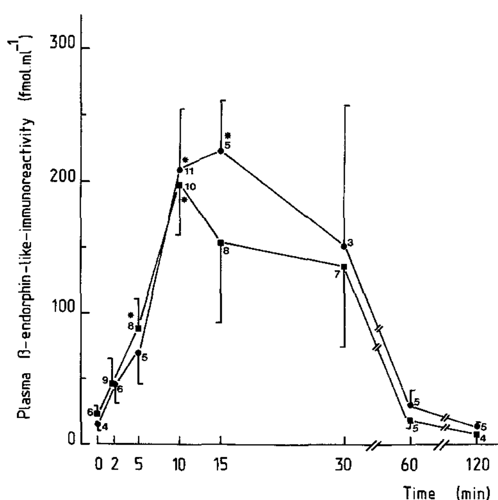

Fig. 1. Time-course of ether stress-induced rise in plasma β-EI. Each point is the mean ± SEM: figures on the graph denote number of determinations in WKY (■) and SHR (●). Asterisks indicate significant changes of β-EI levels in the two strains as compared to the respective values at 0 min ($P < 0.05$)

Statham P23 Db transducer and a Rikadenki R-20 pen recorder. β-E was administered extraluminally in a volume of 4 µl or 12 µl when responses to stimulation had become stable (within about 30 min). Increasing concentrations of the opioid were applied every 24 min for 3 min, during which time two contractions occurred. Depression of vasoconstriction was always measured at its maximum, irrespective of the drug contact-time, and was expressed as percent of the vasoconstriction before addition of the respective agonist concentration. The IC_{50} values, i.e. the concentration that produced 50% of the maximal inhibition obtained with β-E, was calculated by linear regression analysis after probit transformation.

Materials. The following drugs and substances were used: human β-E (UCB Bioproducts, Brussels, Belgium); α-MSH (Bachem, Bubendorf, Switzerland); anti-β-E antiserum (generously provided by W. Knepel, Freiburg i.Br., FRG); anti-α-MSH antiserum (generously provided by Eberle, Basel, Switzerland); ACTH radioimmunoassay kit; ACTH-PR (Oris Industrie, Gif-sur-Yvette, France); Aprotinin (Laboratoire Choay, Paris, France).

Statistical evaluations. Mean ± SEM are given throughout. Student's *t*-test was used to test significance of differences between means.

Results

Effects of ether stress on plasma levels of β-EI, ACTH and α-MSH

The time-course of the effect of 1 min ether stress on plasma β-EI in WKY and SHR is shown in Fig. 1. After stress the plasma levels of β-EI increased rapidly with time and reached peak values between 10−15 min in both strains and thereafter fell towards basal values which were reached after 60 min. No significant difference could be detected in the pattern of β-EI secretion in response to stress between SHR

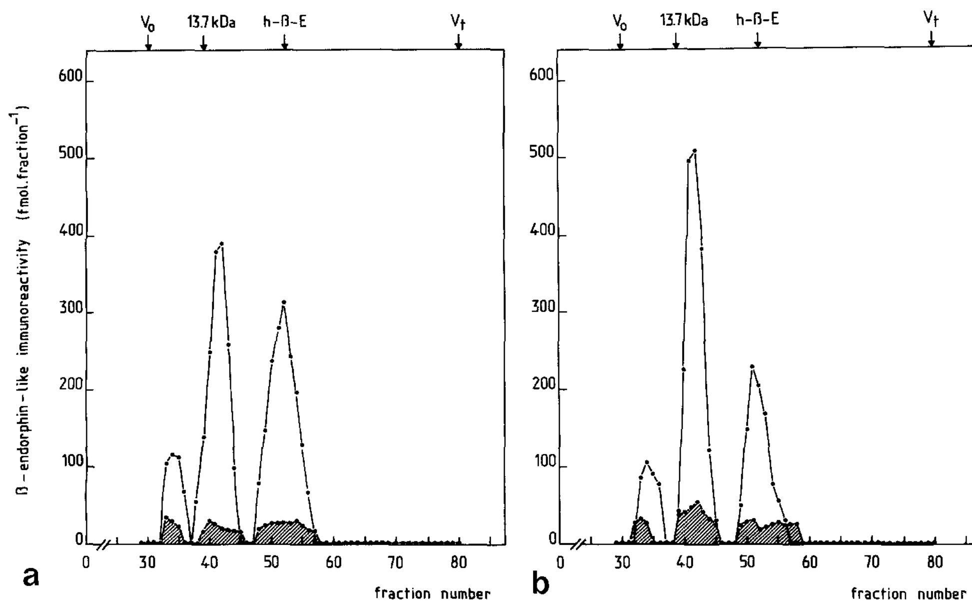

Fig. 2
Gel filtration profiles of β-EI (Sephadex G-50, superfine column) of pooled plasma extracts from WKY (**a**) and SHR (**b**). Results in both unstressed (*hatched area*) and stressed animals are shown. The volume of pooled plasma was 50 ml in unstressed and 26 ml in stressed WKY. The respective plasma volume in SHR were 60 ml and 28 ml. The elution positions for void volume (V_o), the protein marker ribonuclease (13.7 kDa), human β-endorphin (h-β-E) and the total volume (V_t) are indicated by arrows

and their normotensive controls, although after 15 min plasma levels of β-EI seemed to be slightly lower in WKY than in SHR. In subsequent experiments blood samples were always collected 10 min after the beginning of the 1 min ether stress. In view of the appreciable cross-reactivity of β-lipotropin (β-LPH) with the anti-β-E antiserum, gel filtration on a Sephadex G-50 superfine column was performed in order to separate the different molecular forms of circulating β-EI. The gel filtration elution profiles of plasma pools from WKY both under unstressed conditions and in response to ether stress are presented in Fig. 2a; plasma extracts of SHR exhibited a similar elution profile (Fig. 2b). Chromatographic analysis of pooled plasma extracts of both strains showed three β-EI peaks. Two major peaks were found: one eluted after a 13.7 kDa marker protein, and might presumably represent β-LPH (11.7 kDa), the other eluted concomitantly with human β-E. A minor peak eluted at the void volume and may represent the release of the large molecular weight precursor pro-opiomelanocortin. No significant amounts of immunoreactivity were associated with any molecule having a molecular size other than those described here. Ether stress not only elevated plasma levels of β-E, but also those of β-LPH and the large molecular weight component eluted in the void volume. The data in Table 1 indicate that the basal concentration of β-EI was similar in WKY and SHR, whereas WKY had higher levels of ACTH and lower basal levels of α-MSH than SHR. Ten minutes after an exposure to ether stress, plasma levels of β-EI and ACTH were enhanced to the same extent both in WKY and SHR. By contrast, at this time α-MSH levels were not significantly increased either in WKY or SHR.

Table 1. Changes in plasma levels of β-EI, ACTH and α-MSH after ether stress (see Methods). Values given are means ± SEM of (n) animals

		β-EI (fmol · ml^{-1})	ACTH (fmol · ml^{-1})	α-MSH (fmol · ml^{-1})
WKY	Unstressed	91.7±13.0 (14)	25.6± 2.2 (12)	25.2±2.0 (14)
	Stressed	200.9±28.1** (16)	260.5±34.3** (13)	38.8±6.5 (16)
SHR	Unstressed	79.6± 9.3 (13)	16.8± 1.0* (15)	37.4±3.4* (13)
	Stressed	236.5±32.4** (18)	216.1±31.1** (17)	53.1±7.0 (19)

* $P < 0.01$ as compared to WKY of the same group
** $P < 0.001$ as compared to unstressed values

Depression of nerve stimulation-induced vasoconstriction by β-endorphin in the rat tail artery

Vasoconstriction was measured 1.5 h after setting up rat isolated tail arteries (1 h incubation and 30 min stimulation). At this time it did not differ significantly in organs obtained from WKY (18.0 ± 2.1 mmHg, n = 18) and SHR (23.0 ± 2.2 mmHg, $n = 18$, $P > 0.05$). β-E depressed responses to stimulation in a concentration-dependent manner; the maximum inhibition was about 60% in both strains (Fig. 3). The concentration-response curves, and the IC_{50} values derived from these curves (WKY: 145.3 ± 9.1 nmol · l^{-1}, n = 6; SHR: 173.0 ± 15.9 nmol · l^{-1}, n = 6, $P > 0.05$) were also similar.

Discussion

In this paper we describe the effect of acute stress on plasma β-EI, ACTH and α-MSH levels in normotensive and hypertensive rats. Ether breathing, which is a widely used type of experimental stress, increased plasma levels of β-EI and ACTH to a similar extent both in WKY and SHR. The peak values were reached within approximately 15 min after the beginning of the stress in accordance with previous findings (Usategui et al. 1976; Guillemin et al. 1977; Rossier et al. 1977). It has already been shown that ACTH, β-E

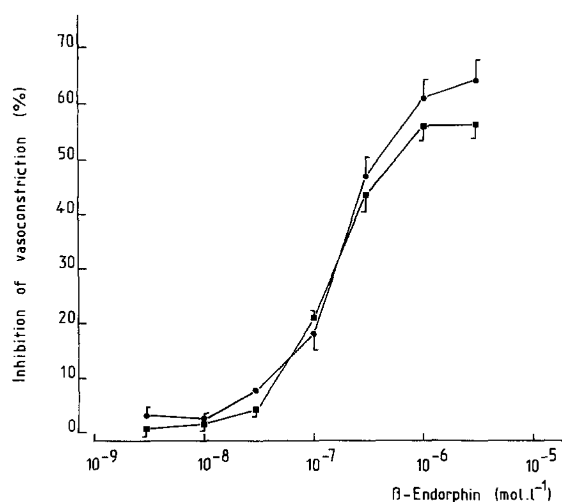

Fig. 3. Concentration-response curves of the effect of β-E on stimulation-induced vasoconstriction in WKY (■) and (●) tail arteries. The preparations were incubated and perfused. Two pulses (0.3 ms, 200 mA) were delivered every 2 min at 1 Hz. Each point represents the mean ± SE of 6 arteries

and several closely related peptides co-exist within the same hypophyseal cells of the rat (Pelletier et al. 1977), and that their secretion varies concomitantly in response to acute stress or secretagogues (Guillemin et al. 1977; Rossier et al. 1977). Thus, our results are in good agreement with these observations. The absence of a significant difference between resting plasma α-MSH values and values measured 10 min after beginning of the ether stress is in keeping with previous observations obtained in normotensive Wistar rats (Usategui et al. 1976). These authors found a transient increase in the plasma concentration of α-MSH, which had already returned to basal values at the time (10 min after the beginning of ether stress) when our measurements were carried out.

Plasma β-EI is known to consist of two major components resembling in molecular weight β-LPH (11.7 kDa) and β-E (3.4 kDa) as revealed by Sephadex chromotography (Anhut et al. 1981; Sapun-Malcolm et al. 1986). Since no β-LPH standard was available we used ribonuclease (molecular weight: 13.7 kDa), as a molecular size marker in the chromatographic separation. The major peak (approximate molecular weight 12 kDa), which eluted after the ribonuclease is most probably β-LPH; not other protein of comparable molecular weight was eluted.

Under in vitro conditions anterior lobe cells release ACTH and two major forms of β-EI resembling β-LPH and β-E in molecular size (Vermes et al. 1980). By contrast, cells of the neurointermediate lobe secrete α-MSH and a single form of β-EI corresponding to β-E in molecular size (Vermes et al. 1980). Since in vitro β-LPH is released only from the anterior and not from the intermediate lobe, plasma levels of an immunoreactive protein of similar size to β-LPH may indicate that β-EI orginates from the anterior lobe in vivo (Höllt and Bergmann 1982; Sapun-Malcolm et al. 1986). In our experiments, elution profiles of β-EI revealed that ether stress elevated plasma levels of immunoreactive proteins of similar size to β-LPH and β-E. Therefore, it appears that after stress the major portion of total plasma β-EI is secreted by the anterior lobe corticotrophs, although a contribution from the intermediate lobe cannot be excluded. The concomitant significant elevation of ACTH also suggests that β-E is of anterior pituitary origin. This β-E is biologically active, in contrast to an α-N-acetylated form of β-E in the intermediate lobe (Smyth et al. 1979).

Although β-EI concentrations in the neurointermediate lobe of the pituitary of SHR (Hutchinson et al. 1981) and stroke-prone SHR (Gaida et al. 1985) were higher than in normotensive controls, this neuropeptide abnormality was probably not related to the development of hypertension (Gaida et al. 1985). In complete agreement with this view, in the present experiments both the basal and stress-induced increase of β-EI levels were similar in SHR and WKY.

Some authors believe assume that the therapeutic effect of clonidine in hypertension is exerted via the release of β-E both, from the pituitary (Pettibone and Mueller 1981; Farsang et al. 1984) and from terminals of opioidergic neurones in the brain stem (Farsang et al. 1980; Kunos et al. 1981). However, neither the experimental data nor their interpretation is undisputed. In a number of laboratories the increase in plasma β-E by clonidine (Yasunari et al. 1985) or the blockade of the cardiovascular effects of clonidine by naloxone (Watkins et al. 1980; Shropshire and Wendt 1983) could not be reproduced.

Since the plasma concentration of β-EI did not differ in WKY and SHR, we determined the sensitivity of presynaptic ε-receptors in tail arteries of the two strains towards β-E. It has been found previously that the release of both endogenous (Galloway and Westfall 1982) and labelled noradrenaline (Zsoter et al. 1982) is higher from caudal arteries of SHR than WKY. This has been suggested to be due both to a supersensitivity of presynaptic facilitory receptors (e.g. angiotensin: Westfall et al. 1985) and a subsensitivity of presynaptic inhibitory receptors (e.g. α_2: Galloway and Westfall 1982; see Westfall and Meldrum 1985). In addition, the maximum contractile responses to both nerve stimulation and exogenous noradrenaline were larger in tail arteries of SHR than WKY (Medgett et al. 1984). It has been proposed that in contrast to WKY, postsynaptic α_2-adrenoceptors may also be involved in vasoconstriction induced by stimulation of the sympathetic nerves in the arteries of SHR (Medgett et al. 1984).

In the present experiments we used a low frequency of 1 Hz and only 2 pulses; this stimulation pattern induced a slightly, but not significantly larger vasoconstriction in tail arteries of SHR than WKY. Apparently the difference becomes more pronounced only at higher frequencies and longer strains of stimuli (Medgett et al. 1984). The potency of β-E in depressing the contractile responses was similar in both strains. Moreover, much higher concentrations of β-E were needed to activate presynaptic ε-receptors, than the available plasma levels.

In conclusion, basal and stress-induced levels of β-EI did not differ in SHR and WKY. Moreover, in the tail artery of both strains the sensitivity of presynaptic opioid ε-receptors towards β-E was almost identical. If the β-E sensitivity of these receptors in other ateries of WKY and SHR is also similar, a major role of the circulating peptide in the development of hypertension is rather unlikely.

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