Vasopressin Is not Involved in the Catecholamine-Induced Release of ACTH, α-MSH and β-Endorphin from the Rat Pituitary Gland

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Abstract. The effect of catecholamines on plasma levels of immunoreactive ACTH (ACTH1), α-MSH (α-MSH1), β-endorphin (β-END1), arginine-vasopressin (AVPi) and of corticosterone (B) was studied in female rats. Intravenous infusion of the specific β-adrenoceptor-stimulating agent l-isoproterenol in Wistar rats under pentobarbital anesthesia resulted in a dose-dependent (dose range: 10–100 ng/kg·min) increase in plasma B. At higher concentrations, l-isoproterenol also caused a dose-dependent increase in plasma AVPi (dose range: 100–1,000 ng/kg·min). In addition to isoproterenol, also intravenous infusion of l-epinephrine caused a dose-dependent increase of plasma B (dose range: 100–1,000 ng/kg·min), whereas l-epinephrine was without effect on plasma AVPi, even at the highest dose tested (1,000 ng/kg·min). The effect of l-epinephrine or l-isoproterenol on plasma B was associated with a parallel and dose-related increase in plasma ACTH1, β-END1 and α-MSH1. The increase of plasma ACTH1, B and β-END1 in response to l-isoproterenol (300 ng/kg·min) was identical in Wistar, Long Evans and Brattleboro rats (Long Evans rats with a hereditary lack of vasopressin). Also the responses to l-epinephrine (1,000 ng/kg·min) were identical in Wistar and Brattleboro rats. We conclude that vasopressin does not mediate the catecholamine-induced release of ACTH, β-endorphin and α-MSH.

Administration of small amounts of epinephrine into the peripheral circulation of rats can stimulate the secretion of α-MSH and β-endorphin from the melanotrophs of the intermediate lobe and ACTH from the corticotrophs of the anterior lobe [1]. The effect of epinephrine on plasma ACTH, α-MSH and β-endorphin is mediated by β-adrenoceptors since it can be mimicked by l-isoproterenol and blocked by l-propranolol [1, 19]. Results of studies with rats bearing hypothalamic lesions support the view that the β-adrenoceptor-mediated effect of catecholamines on ACTH and corticosterone (B) secretion require an intact hypothalamo-hypophysial connection [24], whereas the effect on α-MSH and β-endorphin from the melanotrophs is due to a direct action of catecholamines with β-adrenoceptor sites present on the melanotroph cells [2, 20].

Recently, peripheral administration of l-isoproterenol was found to enhance plasma vasopressin levels in the rat [10]. Also this effect appeared to be mediated by β-adrenoceptors since it could be antagonized by propranolol [10].

Since vasopressin itself exhibits ACTH-releasing activity in vivo and in vitro and can potentiate the ACTH-releasing activity of certain other factors present in median eminence extracts of rats [6] and of synthetic ovine CRF α1 [5, 22], it was of interest to study the possibility that the catecholamine-induced release of ACTH and related peptides is mediated by vasopressin.

Materials and Methods

Animals

Adult female rats were used of different strains: Wistar rats, Long Evans rats (LE) and homozygous Brattleboro rats [LE rats with a hereditary diabetes insipidus (DI)] had a body weight of 140–160 g. 2 rats were housed per cage under a 12:12 h light:dark regimen in which the light periods started at 7.00 a.m. Food and water were available ad libitum.

Handling

The rats were handled and adapted to intraperitoneal injection by administration of 1 ml of saline (0.9% NaCl in water) twice daily for 3 consecutive days prior to the experiment.

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Experimental Protocol

On the day of the experiment, animals were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and the body temperature was maintained at 37 ± 1 °C. A cannula was inserted into a lateral tail vein and vehicle (saline containing 0.1 mM ascorbic acid, pH 7.4) or catecholamine-containing vehicle was infused at a rate of 0.16 ml/min for 20 min, unless otherwise stated. At the end of the infusion period, the animals were decapitated and trunk blood was collected in heparin-containing centrifuge tubes, centrifuged (5 min; 1,000 g; 4°C) and plasma was removed and stored at −20 °C.

Extraction

Plasma aliquots (1.0–1.5 ml) were extracted by using Vycor glass powder (Corning Glass Works, Philadelphia, Pa.) [4, 17]. The extraction efficiency of synthetic human β-endorphin and ACTH1-39 added to rat plasma was 75.0 ± 1.2% (n = 17) and 49.5 ± 3.5% (n = 10), respectively. The extraction efficiency of synthetic arginine-vasopressin (AVP) added to plasma of DI rats was 69.5 ± 6.5% (n = 167). The data on β-endorphin, ACTH and AVP have been corrected accordingly.

Radioimmunoassays

β-Endorphin Immunoreactivity (β-ENDi). β-ENDi was quantitated by a radioimmunoassay as described previously [12, 25]. The antiserum was obtained from Bio-flex Laboratories Inc. (Jamaica, N.Y.), synthetic human β-endorphin, kindly supplied by Dr. H. M. Greven (Organon International BV, Oss, The Netherlands), was used for labeling and as a standard. In this assay human β-endorphin and β-LPH are measured with equimolar efficiencies. Also rat β-endorphin and β-LPH are measured but exact cross-reactivities are not known.

ACTH Immunoreactivity (ACTH). ACTH was determined by a radioimmunoassay as described elsewhere [3, 19]. The antiserum was a gift from Dr. Tj.B. van Wimersma Greidanus (Utrecht, The Netherlands), synthetic human ACTH1-39, kindly supplied by Dr. W. Rittel (Ciba-Geigy, Basel, Switzerland), was used for labeling and as standard.

α-MSH Immunoreactivity (α-MSHi). α-MSHi was determined by a radioimmunoassay as described by Penny and Thody [14]. The antiserum was a gift from Dr. A.J. Thody (Newcastle upon Tyne, England), synthetic α-MSH, kindly supplied by Dr. W. Rittel (Ciba-Geigy), was used for labeling and as a standard.

 Vasopressin Immunoreactivity (AVPi). AVPi was determined by using a radioimmunoassay as described elsewhere [4]. Synthetic AVP (Organon, Oss, The Netherlands) was used for labeling and as a standard.

Corticosterone Assay

The concentration of corticosterone in plasma samples was measured by a fluorimetric assay according to Glick et al. [7] and is designated as plasma B.

Drugs

The following drugs were used: l-isoproterenol-sulfate and l-epinephrine-tartrate (OPG, Utrecht, The Netherlands).

Statistics

Results are presented as mean ± SEM and data were compared by using Student’s t test or Duncan’s multiple-comparison test.

Results

The effect of intravenous infusion of catecholamines on the plasma concentrations of ACTH, B, α-MSH, β-ENDi or AVPi was studied in Wistar, LE and DI rats under nembutal anesthesia. Infusion of vehicle did not affect the plasma concentrations of ACTH, B, α-MSH, β-ENDi and AVPi compared to levels found in plasma of unanesthetized rats. As illustrated in figure 1, infusion of l-isoprote
Table 1. Effect of l-epinephrine on the plasma concentration of ACTH, αMSH, β-ENDi and of B in handled female Wistar and Brattleboro (DI) rats under pentobarbital anesthesia

<table>
<thead>
<tr>
<th></th>
<th>ACTH, ng/ml</th>
<th>αMSH, ng/ml</th>
<th>β-ENDi, ng/ml</th>
<th>B, μg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.19 ± 0.05</td>
<td>0.15 ± 0.01</td>
<td>0.22 ± 0.03</td>
<td>12.2 ± 2.5</td>
</tr>
<tr>
<td>EPI</td>
<td>0.53 ± 0.07</td>
<td>0.28 ± 0.03</td>
<td>1.01 ± 0.14</td>
<td>41.5 ± 2.3</td>
</tr>
<tr>
<td>DI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.10 ± 0.02</td>
<td>0.19 ± 0.02</td>
<td>0.19 ± 0.02</td>
<td>17.9 ± 1.8</td>
</tr>
<tr>
<td>EPI</td>
<td>0.49 ± 0.06</td>
<td>0.32 ± 0.02</td>
<td>0.93 ± 0.10</td>
<td>39.5 ± 1.8</td>
</tr>
</tbody>
</table>

Rats were infused for 20 min with vehicle (control) or 1,000 ng/kg·min l-epinephrine-tartrate (EPI). Data represent mean ± SEM (n = 7 or 8).

proterenol at a dose of 300 ng/kg·min caused an increase in the plasma concentrations of ACTH i, B, α-MSH and β-ENDi. The l-isoproterenol induced increase in ACTH i, B and β-ENDi was identical in Wistar, LE and DI rats (fig. 1). The l-isoproterenol-induced increase in plasma α-MSH in LE rats was less (p < 0.01, Duncan) than in Wistar and DI rats. Although the reason for this smaller α-MSH response to l-isoproterenol in LE rats remains unclear, it is worth noting that the α-MSH content of the neurointermediate lobe of the LE, Wistar and DI rats was 0.81 ± 0.04 μg (n = 7), 1.06 ± 0.06 μg (n = 8), 1.22 ± 0.07 μg (n = 8), respectively.

Infusion of l-epinephrine at a dose of 1,000 ng/kg·min also resulted in a substantial increase in the plasma concentrations of ACTH i, B, α-MSH and β-ENDi. The circulating concentrations of ACTH i, β-ENDi, α-MSH i and B achieved in response to this dose of l-epinephrine were not different (p > 0.2) in Wistar and DI rats (table 1).

The effect of different doses of l-isoproterenol and l-epinephrine on the plasma concentrations of B and of AVPi in Wistar rats is illustrated in figure 2. Infusion of l-isoproterenol caused a dose-related increase in plasma B (dose range: 10–100 ng/kg·min). Infusion of l-isoproterenol also caused an increase in plasma AVPi concentrations, but approximately 10 times higher doses were needed.

Infusion of l-epinephrine also resulted in a dose-dependent increase in plasma B concentrations (dose range: 100–1,000 ng/kg·min). In contrast to l-isoproterenol, l-epinephrine was without effect (p > 0.02) on plasma AVPi concentrations, even at the highest dose tested. Since it was considered that the possible effects of l-epinephrine on plasma AVPi concentrations may have vanished within 20 min, we studied the time course of l-epinephrine on circulating AVPi concentrations. However, the high dose of l-epinephrine (1,000 ng/kg·min) was found to be without effect on the plasma AVPi concentrations, 2, 5 or 10 min after the start of the infusion (p > 0.2).

Fig. 2. Effect of intravenous infusion of different doses of l-isoproterenol-sulfate (a) and l-epinephrine-tartrate (b) on the plasma concentrations of B. ( ● ; μg/100 ml) and AVPi ( ○ ; pg/ml) in handled female Wistar rats under pentobarbital anesthesia. Data represent mean ± SEM (n = 7 or 8). p values: catecholamine-infused rats versus vehicle-infused rats. *Nonsignificant (p > 0.02); **p < 0.01 Student’s t test.)
Discussion

The present study shows that intravenous infusion of \(l\)-isoproterenol to rats under anesthesia causes a dose-dependent increase in the plasma concentrations of AVPi. A similar effect has been demonstrated after intramuscular administration of isoproterenol (bolus injection) to conscious rats [10]. Although \(\beta\)-adrenoceptors appear to be involved in the isoproterenol-induced AVPi secretion, as yet, little is known about the mechanisms involved in this AVPi response.

Catecholamines such as epinephrine or norepinephrine have been reported to stimulate AVPi release from neural lobes and isolated median eminence tissue in vitro, but these effects are mediated by \(\alpha\)-adrenoceptors rather than by \(\beta\)-adrenoceptors [13]. Angiotensin II, which has been shown to stimulate AVPi release in vivo [11], may be involved since its circulating concentration increases after administration of isoproterenol [16]. In addition, the effect of catecholamines on blood pressure might mediate the effects on AVP levels. It is well known that a fall in blood pressure is a powerful stimulus for vasopressin release. Indeed, Knepel et al. [10] reported a close correlation between the increase of plasma AVPi levels and the fall in mean arterial blood pressure after intramuscular administration of isoproterenol. A fall in systolic and diastolic blood pressure can also be observed under our experimental conditions in response to isoproterenol [19]. The role of hypotension in AVPi release under these conditions is further supported by the observation that epinephrine, which can interact with \(\alpha\)-and \(\beta\)-adrenoceptors, induces an increase in systolic blood pressure [19] and does not affect AVPi plasma concentrations (fig. 2).

We have reported earlier that intravenous infusion of \(l\)-isoproterenol to anesthetized rats causes an activation of the pituitary-adrenal system [19] which was recently confirmed by Knepel et al. [9] by intramuscular administration to conscious rats. This effect of isoproterenol on the activity of the pituitary-adrenal system appears to be mediated by \(\beta\)-adrenoceptors since it can be mimicked by epinephrine and is antagonized by \(l\)-propranolol but not by \(d\)-propranolol [19]. However, the site of action of isoproterenol on the pituitary-adrenal system is poorly defined.

Catecholamines have been reported to stimulate the release of peptides from cultured corticotrophs; however, this effect is mediated by \(\alpha\)-adrenoceptors rather than \(\beta\)-adrenoceptors [15, 18, 23]. It is reported that anterolateral deafferentation of the mediobasal hypothalamus induces a disappearance of CRF-immunostainable neurons in the median eminence [21] and prevents the \(\beta\)-adrenoceptor-mediated pituitary adrenal activation [24]. However, since this lesion also interrupts vasopressinergic fibers projecting to the median eminence and posterior lobe, the possibility that vasopressin plays a role in the isoproterenol-induced activation of the pituitary-adrenal system, suggested by Knepel et al. [9], could not be excluded. The results of the present study are in disagreement with this possibility. Firstly, doses of isoproterenol that are ineffective in elevating circulating AVPi (e.g. 10 ng/kg·min) can activate the pituitary-adrenal system. Secondly, isoproterenol and epinephrine both stimulate pituitary-adrenal activity via a \(\beta\)-adrenoceptor mechanism [19], but only isoproterenol elevates plasma AVPi levels (fig. 2). Thirdly, the increase of plasma ACTHI and B concentrations following infusion of isoproterenol is identical in rats congenitally lacking vasopressin (DI rat) and in normal rats (LE and Wistar rats).

In addition to AVPi, ACTHI and B, \(\beta\)-adrenoceptor stimulation also increases the secretion of \(\alpha\)-MSH and \(\beta\)-ENDi [1]. In fact, the medial effective doses of \(l\)-epinephrine and \(l\)-isoproterenol for stimulating ACTHI, B, \(\alpha\)-MSH and \(\beta\)-ENDi secretion are identical [1]. Studies with hypothalamic lesions and recent studies, in which peptide secretion from either the corticotrophs or the melanotrophs is selectively suppressed, revealed that, under our experimental conditions, most of the \(\beta\)-ENDi secreted in response to catecholamines originates together with \(\alpha\)-MSH from the melanotrophs [1, 24]. This is in disagreement with conclusions of Knepel et al. [8], that may be based on a misinterpretation of their results since the secretion of \(\alpha\)-MSH and other peptides from the intermediate lobe can be inhibited by dexamethasone in doses as low as 250 \(\mu\)g/kg s.c. for at least 2 h [Berkenbosch et al., in prep.]. The effect of catecholamines on \(\alpha\)-MSH and \(\beta\)-ENDi secretion is most likely due to a direct interaction of the catecholamines with \(\beta\)-adrenoceptor sites that are known to be present on the intermediate lobe cells [2, 20]. In conclusion, our data strongly support the view that vasopressin does not mediate the release of ACTH, \(\beta\)-endorphin and \(\alpha\)-MSH following \(\beta\)-adrenoceptor stimulation.

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