Gender-dependent characteristics of the hypothalamo-corticotrope axis function in glucocorticoid-replete and glucocorticoid-depleted rats

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ABSTRACT. The aim of the present study was to determine the role of the endogenous sex steroid environment in the hypothalamo-corticotrope (HC) function in both sham-operated (SHAM) and bilaterally adrenalectomized (ADX) rats. For this purpose adult rats of both sexes were used 3 and 6 weeks after either SHAM or ADX. The results indicate that: a) in SHAM animals, basal plasma ACTH levels were significantly higher in females than in males, and this sexual dimorphism was overridden by ADX, regardless of the time post-surgery; b) although basal anterior pituitary (AP) ACTH content was similar in SHAM animals of both sexes, 3- and 6-week ADX induced higher AP ACTH in males than in females; c) at 3- and 6-weeks, ADX rats of hoth sexes had an AVP:CRH ratio (r), in the median eminence (ME) and medial basal hypothalamus (MBH), increased several fold over the respective SHAM-value and, although no sexual dimorphism was found at week 3 post-ADX, by 6-weeks post ADX, these ratios were significantly higher in both brain tissues of females than in those of males; and d) the *in vitro* ME CRH and AVP output in response to high potassium concentrations (hK^+; 28 and 56 mmol/l), was concentration-related, regardless of sex and surgery, and was characterized by enhanced secretion of neuropeptides by MEs from ADX in comparison to SHAM rats of both sexes, and a sexual dimorphism was found in this parameter, consisting in general, in greater neuropeptide output from tissues of female than of male animals. Our results indicate that: 1) there is a gender-dependent characteristic of the HC axis function in glucocorticoid-replete rats and 2) the absence of the glucocorticoid negative feedback mechanism is responsible for either the expression or for the override of the sexual dimorphism in different parameters, a phenomenon which dependent on the time elapsed after ADX.

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INTRODUCTION

Hypothalamo-pituitary-adrenal (HPA) axis function is the result of different feedback mechanisms at the three levels of the neuroendocrine system (1). CRH and arginine-vasopressin (AVP) are the major mediators of anterior pituitary (AP) ACTH synthesis and secretion (1). In the paraventricular nucleus (PVN) of the hypothalamus, CRH and AVP are co-localized in parvicellular neurons that project to the external zone of the median eminence (ME) (2). Hence, these neuropeptides are released into the portal blood and reach the AP, thus controlling corticotrope function (3). The surgical ablation of the adrenal glands (ADX) induces an increase in plasma ACTH levels and this effect is known to be mediated by stimuli from the PVN (4).

Although the degree of participation of CRH and AVP on ACTH production following ADX has been investigated (5), and studies on the sexual dimorphism of the HPA axis function in basal and following different stress stimuli have been published (6-9), data on the influence of the endogenous sex steroid environment on ADX-induced changes in the function of the hypothalamic CRH and AVP neuronal pathways are still lacking. The present study, determined the sex-dependent characteristics of the hypothalamo-corticotrope (HC) function in glucocorticoid-replete as well as in 3- and 6-week-adrenalectomized rats.

Key-words: Adrenalectomy, CRH, AVP, ACTH, neurosecretion, sex steroids.

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MATERIALS AND METHODS

Experimental design

Adult Sprague-Dawley male (280-330 g body weight) and female (240-280 body weight) rats were subjected to bilateral adrenalectomy (ADX) or sham operation, under ether anesthesia, by the dorsal approach. ADX rats were supplied with water containing 0.9% NaCl. Animals were housed individually and killed by decapitation (between 08:00 and 09:00 hours) (10) 3 and 6 weeks after operation. Trunk blood was collected into plastic tubes containing 0.5 ml of EDTA (10%, w/v) and plasma samples were kept frozen (-20 C) until ACTH concentrations were determined. Immediately after decapitation, brain tissues were quickly removed and the AP, the median eminence (ME) and the medial basal hypothalamus (MBH) were dissected as previously described (11). Tissues were sonicated in an appropriate volume of HCl 0.1 mol/l and centrifuged, supernatants were kept frozen until measurement of AP ACTH as well as CRH and AVP in both the ME and the MBH.

In additional experiments, different groups of rats were killed (between 08:00 and 09:00 hours) (10) on different weeks (3 and 6) after SHAM or ADX and the MEs were dissected for *in vitro* experimentation. Briefly, 10 MEs from each experimental group were packed in a column and superfused (8-min pulses, 0.3 ml/min flux rate) as previously described (11) with medium (Earle's balanced salt solution containing BSA 2 g/l, Trasylol 100 IU/ml and antibiotics) alone (5 mmol/l KCl) or containing a high concentration of KCl (hK^+; 28 and 56 mmol/l). Medium CRH and AVP concentrations were determined on the 8-min fractions collected.

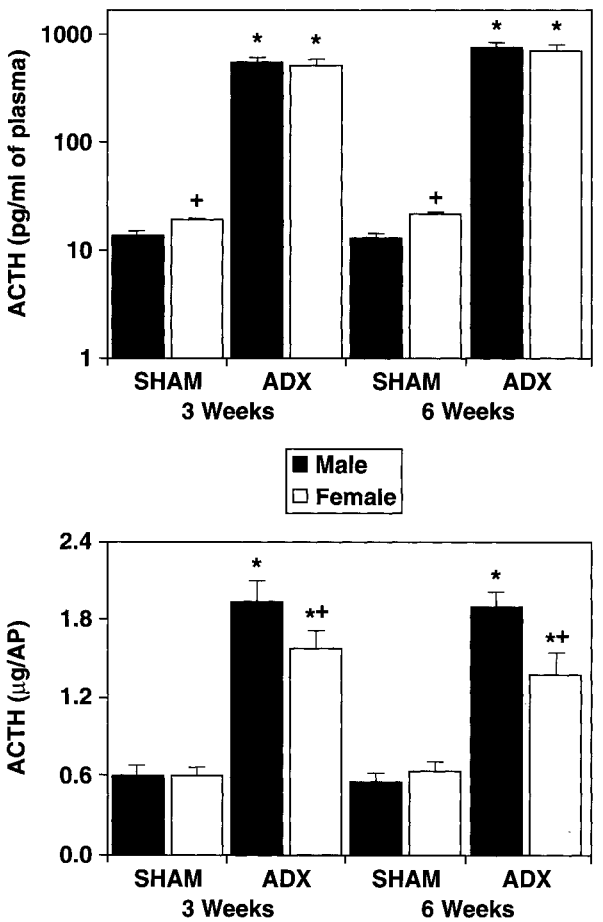

Fig. 1 - *Plasma (upper panel) and anterior pituitary (lower panel) ACTH concentrations in sham and adrenalectomized rats of both sexes. Results are the mean±SE of 8-12 rats per group.*
**,p<0.05 or less vs the respective sham-values.*
+, p<0.05 vs the respective male-values.

Hormone assays

ACTH. Plasma ACTH and AP ACTH concentrations were determined by a two-site immunoradiometric assay as reported in detail (12). The intra-and inter-assay coefficients of variation were 2 and 6%, respectively. Neuropeptides. Tissue and medium concentrations of CRH (13) and AVP (14) were determined by specific radioimmunoassays as previously reported. The intra- and interassay coefficients of variation ranged between 5-8 and 10-12%, respectively.

Statisticals

Results are expressed as the mean±SE. Data were analyzed by multifactorial analysis of variance followed by Fisher's test for comparison of different mean values (15).

RESULTS

Sexual dimorphism in peripheral and anterior pituitary ACTH concentrations

Figure 1 shows plasma (upper panel) and anterior pituitary (lower panel) ACTH concentrations in rats of both sexes and at different periods after surgery. As depicted, basal (SHAM) plasma ACTH levels were in a dimorphic fashion, the female values significantly ($p<0.05$) higher than male values at both 3 and 6 weeks following surgery. As expected, ADX was able to enhance ($p<0.01$) plasma ACTH levels several fold over SHAM values in animals of both sexes, regardless of the time post-operation. The sexual dimorphic pattern of plasma ACTH concentrations observed in SHAM animals was fully abol-

ished by ADX, regardless of time post-surgery (Fig. 1, upper panel).
For AP ACTH, no gender-dependent differences were found in SHAM animals (Fig. 1, lower panel). However, ADX induced a significant ($p<0.05$ or less) increase in AP ACTH over SHAM values, regardless of sex-group and time post-surgery. Interestingly, the increase in AP ACTH had a dimorphic pattern in both periods of time after ADX; a higher ($p<0.05$) AP ACTH was found at both 3 and 6 weeks post ADX in males than in matched female animals.

AVP:CRH relationships (r) in different brain areas at different times after surgery

We have determined AVP and CRH contents in both ME and MBH brain tissues (see Table 1). Thereafter, AVP:CRH r were calculated for each individual and these values (mean±SE), for each brain area, are shown in Figure 2. The upper panel of Figure 2 shows the AVP:CRH r in the ME of SHAM and ADX animals 3 and 6 weeks after surgery. As showed, the ratios in SHAM animals of both sexes were similar. ADX, at both 3 and 6 weeks, induced a significant ($p<0.05$) increase over SHAM values in these ratios; the increase in these ratios were predominantly due to an increase in ME AVP (see Table 1). Although no sex-dependent differences were found at week 3 after ADX, a clear sexual dimorphism in these ratios was found by week 6 post-ADX, the female values being significantly ($p<0.05$) higher than male values. This sexual dimorphic pattern seems to be due to a significant ($p<0.05$ *vs* the respective SHAM value) increase in ME CRH occurred in male animals (Table 1). It must be pointed out that, in females animals, AVP:CRH r at week 6 post-ADX were significantly ($p<0.05$) higher than those found at week 3 post-ADX. The difference in AVP:CRH ratios between weeks 3 and 6 post-ADX were mainly due to a significant ($p<0.05$ *vs* the respective values at week 3 after ADX) increase in ME AVP at week 6 post adrenal ablation, regardless of the sex (Table 1).
Figure 2 (lower panel) shows AVP:CRH r in the MBH of animals of both sexes and on different weeks following surgery. As found for the ME, MBH AVP:CRH r were similar in SHAM animals of both sexes, regardless of the time post-surgery, and were significantly ($p<0.05$) higher than SHAM values as a consequence of ADX, regardless of the experimental week. The increase in the ratios at week 3 post-ADX was fully dependent on a decrease in MBH CRH, regardless of the sex (Table 1). Again, by week 6 after ADX, female AVP:CRH r were significantly ($p<0.05$) higher than both week-matched male and 3-week-ADX female values. This sexual dimorphism was present because female animals shown a significant ($p<0.05$ *vs* the respective male values) increase in MBH AVP (Table 1).

Neuropeptide release by superfused median eminence nerve terminals from glucocorticoid-replete and -depleted rats of both sexes

Figure 3 shows the results of neuropeptide secretion by superfused (8-min pulses) ME terminals of different groups of male (left panels) and female (right panels) rats, with medium alone (Basal, 5 mmol/l KCl) or containing hK^+ (28 and 56 mmol/l). Results are the mean (±SE) of 2 different experiments; since values of 3- and 6-week post SHAM operation were similar, they were pooled and only one SHAM concentration-response curve is shown for each neuropeptide/sex group.

Table 1 - *Median eminence (ME) and medial basal hypothalamus (MBH) CRH (ng per tissue) and AVP (ng per tissue) contents in SHAM and ADX animals of both sexes (M: male F: female). Values are the mean±SE, no.=8-12 rats per group.*

Brain Area / Neuropeptide / Group	ME CRH	ME AVP	MBH CRH	MBH AVP
Week 3				
M SHAM	3.17±0.56	22.62±3.92	5.88±1.16	8.91±1.91
F SHAM	2.90±0.17	23.61±2.02	9.08±1.96	14.06±2.07
M ADX	3.38±0.20	78.61±7.44[1]	2.01±0.76[1]	9.06±0.83
F ADX	2.50±0.18	54.02±3.51[1,3]	2.98±0.55[1]	10.38±1.02
Week 6				
M SHAM	2.98±0.47	24.82±1.25	6.81±0.28	9.78±0.94
F SHAM	3.11±0.31	26.61±2.12	8.09±1.11	12.48±1.17
M ADX	4.63±0.61[1,2]	97.98±6.25[1,2]	1.95±0.24[1]	8.57±0.79
F ADX	3.08±0.42[3]	95.31±5.13[1,2]	1.64±0.25[1]	12.27±1.57[3]

[1],$p<0.05$ *vs* the respective SHAM values.
[2],$p<0.05$ *vs* the respective 3-week-ADX values.
[3],$p<0.05$ *vs* the respective male values.

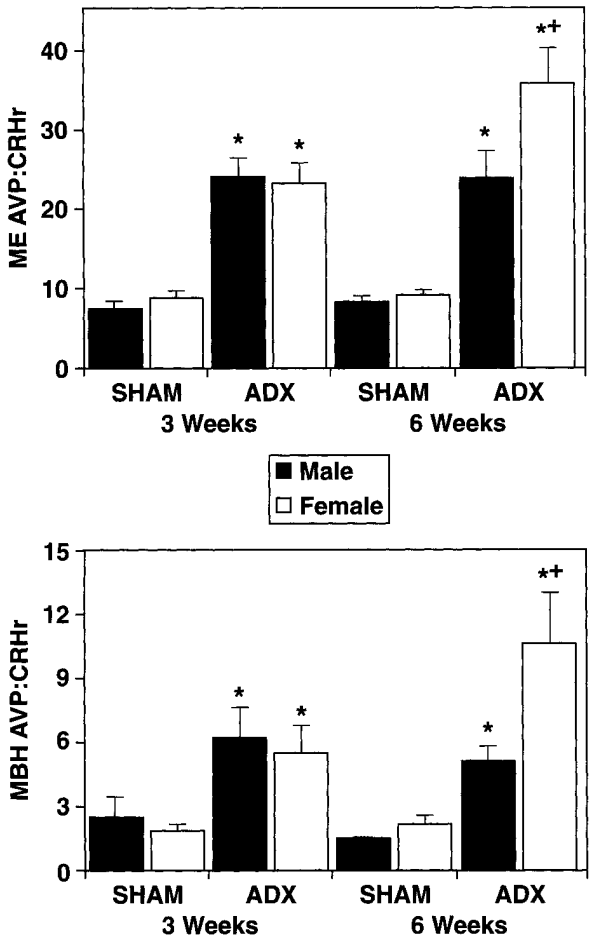

Fig. 2 - *Median eminence (upper panel) and medial basal hypothalamus (lower panel) AVP:CRH ratios in sham and adrenalectomized rats on different weeks after surgery. Results are the mean±SE of 8-12 rats per group.*
**,p<0.05 or less vs the respective sham-values.*
+,p<0.05 vs the respective male-values.

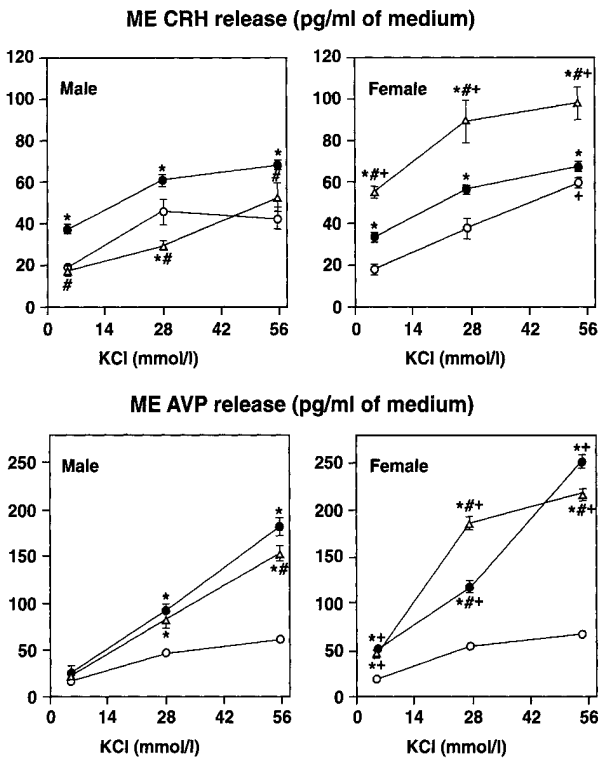

Fig. 3 - *CRH (upper panels) and AVP (lower panels) output by median eminence nerve terminals of sham and adrenalectomized male (left panels) and female (right panels) rats superfused with medium containing physiological (5 mmol/l) or high concentration (28 and 56 mmol/l) of KCl. Results for ADX animals are the mean±SE of two different experiments. Sham results are the mean±SE of two experiments in which one experiment was performed using rats on week 3 and the other on week 6 after operation. SHAM: open circles. 3-week-ADX: closed circles. 6-week-ADX: open triangles.*
**,p<0.05 vs the respective SHAM values.*
#,p<0.05 vs the respective values in 3-week adrenalectomized animals.
+,p<0.05 vs the respective values in male rats.

Figure 3 (upper left panel) shows that basal ME CRH output by MEs from SHAM male rats was significantly ($p<0.05$) enhanced, in a concentration-related fashion, by increasing hK^+ concentrations. Three weeks post-ADX, basal CRH secretion was significantly ($p<0.05$) enhanced *vs* the respective SHAM-values, and neuropeptide output was significantly ($p<0.05$) increased by hK^+. On week 6 after ADX, basal ME CRH release was back to SHAM values and the increase in hK^+ significantly enhanced ($p<0.05$) neuropeptide secretion *vs* the respective basal value. Figure 3 (upper right panel) shows ME CRH release in female groups. The concentration-dependent secretion pattern found in SHAM tissues was significantly ($p<0.05$) enhanced in 3-week post-ADX rats, and at 6 weeks post-ADX, this pattern was at a higher level than those of both 3-week-ADX ($p<0.05$) and SHAM ($p<0.01$) animals. Some sexual dimorphic differences were found in ME CRH output: a) 56 mmol/l KCl-induced CRH output it SHAM rats, with female values significantly ($p<0.05$) higher than male values; and b) basal and hK^+-stimulated neuropeptide secretion in 6-week-ADX animals, female values being significantly ($p<0.05$) higher than male values.

Figure 3 (lower left panel) shows ME AVP secretion by tissues of different groups of male rats. Basal neuropeptide output was unchanced after ADX, regardless of the week, *vs* SHAM response. However, ADX after 3 and 6 weeks significantly ($p<0.05$ or less) enhanced ME AVP output in response to hK^+

vs SHAM values, although there were no significant differences between values of 3- and 6-week post-ADX rats for 28 mmol/l KCl-stimulated AVP release, 56 mmol/l KCl-elicited AVP output was significantly ($p<0.05$) higher in 3- than in 6-week ADX animals. In contrast to findings for males, basal ME AVP output was significantly ($p<0.05$) enhanced *vs* SHAM values by ADX, regardless of the week in females. Additionally, hK^+-stimulated AVP secretion was significantly ($p<0.05$ *vs* SHAM values) enhanced by 3- and 6-weeks post-ADX. As found for ME CRH, ME AVP release was also sexual-dimorphic: a) basal neuropeptide output, in 3- and 6-week-ADX animals, was significantly ($p<0.05$) higher in females than in males; and b) high KCl (28 and 56 mmol/l)-stimulated neuropeptide secretion was significantly ($p<0.05$) higher in female than in male rats at 3 and 6 weeks post-ADX.

DISCUSSION

The present study indicates that: a) there is an *in vivo* sexual dimorphism in basal ACTH release in glucocorticoid-replete rats, the plasma circulating levels being higher in females than in males, b) the loss of the negative glucocorticoid feedback mechanism, at 3 and 6 weeks, induced a persistent increment in ACTH secretion in plasma and overrode the sexual dinorphic pattern of ACTH secretion in plasma; c) ADX, after 3 and 6 weeks, was able to enhance the hypothalamic CRH-and AVP-ergic systems function, whereas the increased neuronal systems function was maximal 3 weeks following ADX in males, while in females, a maximal peptidergic (CRH- and AVP-ergic) function was found 6 weeks post surgical adrenal ablation, thus showing another gender difference in the HC function of rats; d) the enhanced neuronal systems function was not only observed *in vivo* since, *in vitro* ME nerve terminals released, as a consequence of ADX and in general, a higher amount of neuropeptides than SHAM animals, regardless of the sex, and, interestingly, in general, tissues of females were more active than those of male matched-animals.

The sexual dimorphism in plasma ACTH levels found in resting condition corroborate previous results from our laboratory which showed that not only ACTH but also corticosterone plasma levels are significantly higher in adult female than in age-matched male rats of several strains, including Sprague-Dawley (7, 16). However, our results indicate that the loss of a negative feedback glucocorticoid mechanism overrides the sexual dimorphism in plasma ACTH concentrations. It is possible that a very conspicuous mechanism of sex-steroid regulation of hypothalamic ACTH secretagogue-induced ACTH secretion takes place with low concentrations of AVP/CRH in portal blood, however, as soon as the AVP/CRH levels increase in portal circulation as a consequence of ADX (17, 18), this sex-related difference expires. Additionally, a modulatory effect of the endogenous sex-steroid environment on changes in the function of AP CRH- and AVP-binding sites after ADX (19, 20) should not be discarded.

With reference to AP ACTH, a direct correlation was found between this pattern and that of ACTH secretion in plasma at different periods after ADX. Thus indicating that a stable increased AP ACTH synthesis and release can be observed only after several weeks of ADX, a fact which agrees with previous observations (21, 22).

A very interesting sex-related event was observed following ADX. Unlike the response observed in glucocorticoid-replete animals, AP ACTH turnover in glucocorticoid-depleted animals changed in a sex-related fashion, and was characterized by higher AP ACTH content in male than in female rats, regardless of time elapsed after ADX. It remains to be determined whether endogenous sex steroids play any relevant role in this sexual dimorphic difference on a sex-steroid basis, or whether it is simply a gender-dependent characteristic. For example, other researchers described (23) that angiotensin II-induced ACTH release in rats is clearly modulated by the endogenous sex steroid environment, with a lower corticotrope function in ovariectomized than in intact females, and that this effect can be fully reversed by estradiol replacement therapy; these changes were fully due to direct changes in angiotensin II receptor number at the AP level (23), thus clearly indicating a sex-hormone basis for the regulation of the corticotrope function. In addition, sex-dependent characteristics in the sensitivity of the different levels, such as the hyppocampus and/or the corticotrope cell itself, of the hypothalamo-corticotrope unit after the removal of the negative glucocorticoid feedback mechanism should be not ruled out.

Regarding AVP:CRH r in both brain areas, ADX enhanced this parameter over the respective SHAM-values over both 3 and 6 weeks after adrenal ablation. This observation tallies with the results of Holmes et al. (5), who showed that ME AVP:CRHr increased 4 days and 4 weeks after ADX in rats, in the present study, we found that this event is not confined to this brain area but also extends to the MBH. We found that the increase in the ratios was more dependent on AVP increase than on CRH, coinciding with the results of *in vitro* release by MEs in response to depolarizing stimuli. In fact, ADX-induced ME AVP output in response to hK^+ solutions

was increased several fold over SHAM responses on both experimental weeks, however, CRH released in the same conditions was similar or increased, though not to the extent observed for AVP. Therefore, this increase in AVP content in both hypothalamic areas after ADX should have functional significance, since this difference was also observed for *in vitro* ME neuropeptide output. This observation is in agreement with earlier results indicating that at 14 days post ADX there is an increase from 50 [found in basal condition (24)] to 95% (25) of CRF+/AVP+ neuron terminals in the external zone of the rat median eminence. Table 2 shows that AVP secretion, in general, predominated over that of CRH; in fact, AVP:CRHr of output by superfused ME terminals increased somewhat with increasing concentrations of KCl concentrations in SHAM animals of both sexes, however, this increase was several fold higher when examined in adrenalectomized rats on both experimental weeks, regardless of sex. Our results also indicate that ME and MBH AVP:CRHr (in content) of week 6 post-ADX in female animals were significantly ($p<0.05$) higher than in week-matched male rats (see Fig. 2).Thus indicates not only a predominant effect of the loss of the negative glucocorticoid feedback mechanism on AVP-ergic function, but also a possible facilitatory, non-genomic (26) effect of estrogen on hypothalamic AVP synthesis/secretion after long term adrenalectomy. This observation tallies with previous studies indicating that estrogens positively modulate HC function (23, 27, 28) by a mechanism possibly due to an effect of the steroid on glucocorticoid receptor activity (29). Regarding our results for the male sex, it must be pointed out that a negative modulatory role of testosterone on AP ACTH secretion and on ME neuropeptides output should be not ruled out. In fact, it has been reported that androgen decreases HC function by inhibiting readily releasable hypothalamic CRH by an androgen receptor-mediated effect at the PVN level (30), a brain site where a neuronal subpopulation cosynthesizes CRH and AVP (2).

In summary, our data suggest that, aside from a larger role of AVP than CRH in ADX-induced enhanced corticotrope activity, a sexual dimorphism characterizes the HC function in glucocorticoid-replete and -depleted rats. This gender-dependent characteristic indicates that the HC axis of females is more reactive than that of males. It remains to be determined whether this gender-related characteristic is due to a direct effect of sex-steroid hormones.

Table 2 - *AVP:CRH ratios (r) of* in vitro *secretion by superfused median eminence nerve terminals of SHAM and different-week ADX rats, with medium containing physiological (5 mmol/l) or high concentrations (28 and 56 mmol/l) of KCl.*

KCl [Concentration] Group	AVP:CRHr[1] 5 mmol/l	28 mmol/l	56 mmol/l
M SHAM	0.87	0.99	1.43
F SHAM	1.03	1.43	1.11
M 3-week-ADX	0.68	1.49	2.66
F 3-week-ADX	1.52	2.07	3.65
M 6-week-ADX	1.23	2.77	2.89
F 6-week-ADX	0.84	2.07	2.19

[1]values obtained from data in Figure 3.

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