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Effects of glycyrrhizic acid and steroid treatment on corticotropin releasing factor and β-endorphin containing neurons of the hypothalamus of the rat

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Abstract

Corticotrophin releasing factor (CRF) and β-endorphin (βEP) containing neurons are shown to be present in the hypothalamus and both neurons are found at the paraventricular nucleus (PVN). Steroid hormones have been found to alter the plasma level of these neurotransmitters. Glycyrrhizic acid (GCA) is the active component of liquorice. GCA inhibits the enzyme 11β-hydroxysteroid dehydrogenase (11HSD) which is needed for the inactivation of the steroid pathway, so therefore would cause changes to these neurons. The aim of this study was to investigate the effects of GCA as well as deoxycorticosterone (DOC) and dexamethasone (DM) on the modulation of CRF and βEP containing neuron at the PVN of the hypothalamus. Rats were given either DM, DOC or GCA and adrenalectomized (ADX) and given either DM or DOC. At the end of treatment rats were transfused transcardially before sacrifice and the brain were dissected for immunohistochemical analysis. We found that immunostaining of the CRF containing neurons demonstrate a reduction in the number of positive neurons in DM treated rats. DOC and GCA treated rats showed the same result as in DM rats but the reduction is less. ADX, DM, DOC and GCA treated rats did not show any changes in the number of βEPcontaining neurons but naloxone increased the number of βEP containing neurons markedly. In conclusion, GCA and DOC have similar effects on CRF and βEP containing neurons at the PVN.

Key words: Corticotropin releasing factor, β-endorphin, steroid hormones, glycyrrhizic acid, hypothalamus

INTRODUCTION

CRF is the key chemical signal coordinating and integrating the organisms endocrine, autonomic and behavioural responses to stressful and other adaptive stimuli. Immunohistochemical and radioimmunoassay studies have shown CRF to be heterogenously distributed throughout the mammalian central nervous system. Most CRF neurons are in the parvocellular subdivisions of the paraventricular nucleus (PVN) in the hypothalamus.[1-6] Adrenalectomy induces an activation of the CRF neurosecretory system[7] causing increase in basal and stimulated CRF secretion.[8,9] This hypersecretion can be suppressed by glucocorticoid replacement.[10,11] It has been proposed that the brain is the main site of glucocorticoid action to inhibit adrenalectomy-induced ACTH output.[12]

Significant amounts of β-endorphin are found in the hypothalamus.[13] Rats treated with dexamethasone showed a concomitant decrease in plasma ACTH and βEP. These suggest that ACTH and βEP are released simultaneously from the pituitary gland and that the regulatory mechanisms that are involved in the secretion and biosynthesis of both neuropeptides are common and identical.[14]

Glycyrrhizic acid (GCA) the active component of liquorice inhibits the activity of 11β-hydroxysteroid dehydrogenase (11HSD). 11HSD is a microsomal enzyme complex which catalyses the reversible conversion of cortisol to cortisone in man and corticosterone to 11-dehydrocorticosterone in rats. Inhibition of 11HSD results in higher tissue corticosterone or cortisol levels that will act on type I mineralocorticoid receptors in the kidney[15] with resultant sodium retention and hypertension. A wide distribution of 11HSD activity has been described in the brain, namely in the hippocampus, cortex, pituitary, hypothalamus, brain stem and spinal cord.[16] Glycyrrhizic acid also caused an increase in right atrial pressure as

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well as thickening of the pulmonary vessels suggesting pulmonary hypertension.[17] Orally administered GCA is almost completely hydrolyzed by intestinal bacteria and reaches the systemic circulation as glycyrrhetinic acid.[18] Administration of glycyrrhetinic acid decreased CRF release into hypophysial portal blood[19] suggesting that 11HSD regulates the effective corticosterone feedback signal to CRF neurones. Thus GCA may interfere with steroid metabolism in the brain particularly upon the HPA axis. The effect of GCA on the modulation of CRF and βEP containing neurons has not been documented. This study was done as a continuation from previous work done in our laboratory[20] which showed that stress induced hypotension was due to the release of endogenous opioids because this phenomena could be blocked by the pure opioid antagonist: Naloxone. We hypothesized that the endogenous opioid βEP from the hypothalamus is responsible. To ensure the validity of the immunostaining technique we look at the CRF containing neurons. In this study we investigated the effects of glucocorticoid, mineralocorticoid and glycyrrhizic acid on CRF and βEP containing neurons in the PVN of the hypothalamus.

MATERIALS AND METHODS

Animals and experimental treatments

Adult male Wistar rats were divided into 8 groups of 6 rats each. These were control rats, adrenalectomized (ADX) rats, intact and ADX rats treated with either dexamethasone (DM) (Sigma Chemical Co. USA) 120 μg/kg[21] or deoxycorticosterone (DOC) (Sigma Chemical Co. USA) 2.4 mg/kg[21] in 0.1 ml olive oil intramuscularly daily for 7 days, intact rats which received 0.1 ml of naloxone hydrochloride (DuPont Pharmaceutical Inc. Puerta Rico) (0.32 μg/100g) intramuscularly daily for 7 days and rats which received drinking water containing 1.0 mg/ml GCA (Sigma Chemical Co. USA) for 9 weeks. The average amount of water consumed was 40-50 ml per day per rat. ADX rats were given normal saline and intact rats tap water *ad libitum*.

Tissue processing

Prior to sacrifice, rats were injected intraperitoneally with 500 units of heparin then anaesthesized with 25 % urethane (0.6 ml/100 g body weight) intraperitoneally to abolish the tail pinch reflex. The rats were placed on their back and a cut made through the abdominal muscle near the xiphoid process. The xiphoid process was held while the diaphragm was cut. The heart was exposed by cutting through the rib cage along each side and lifting the xiphoid process. A cannula was inserted into the left ventricle and perfused with 200 ml of phosphate buffer (PBS) (0.15M NaCl, 10^{-4}M KH_2PO_4, 10^{-3}M $Na_2HPO_4 12H_2O$, 10^{-3}M $NaNo_3$, pH 7.6, heparin 5000 unit/l) or until the effluent remained clear followed by Bouins fixative (750 ml picric acid, 250 ml 40% formaldehyde, 50 ml glacial acetic acid) for 10-15 minutes. PBS and Bouins fixative were kept at 4°C before and during procedure. When fixation was complete, the rats were decapitated and brains removed. The hypothalamus were dissected, the area near the optic chiasma was marked and placed in Bouins fixative for 1 hour to make sure it was completely fixed and then placed in 10 % sucrose in PBS overnight at 4°C. On the following day the tissue was placed in albumin/gelatin (30%/0.5%) embedding medium for 4 hours, then immersed in 30% sucrose solution overnight at 4°C. The hypothalamic tissue blocks were mounted on metal moulds surrounded with OCT compound and frozen at -20°C, then cut serially on a vibratome at 5 microns. The cutting process was done from the marked optic chiasma area, slices were counted and taken from the same level for every hypothalamic block. Tissue sections were collected on glass slides coated with poly-L-lysine (Sigma Chemical Co. USA). Slides were kept at -20°C until the staining procedure.

Immunohistochemical staining was based on the labelled strepavidin-biotin (LSAB) method using Dako LSAB 2 Kit (USA). The slides were soaked in tris buffer (TBS) pH 7.6 for 30 min to remove the remaining Bouins fixative then kept in 3% hydrogen peroxide for 10 min to suppress endogenous peroxidase activity of the tissue. After a brief rinse with TBS, the sections were exposed to 0.5% normal goat serum in TBS for 20 min to block non-specific back ground staining, excess were then discarded using a pipette. Sections were incubated with the primary antibody, anti-CRF (human, rat) or anti-βEP (human,rat) (Peninsula Lab. USA) diluted in TBS, pH 7.6 with 1% bovine serum albumin for 60 min. The optimum antibody dilution used in this study was 1:1000 for both antibodies. The slides were rinsed and bathed in TBS three times, then incubated with the link antibody, biotinylated anti-mouse and anti-rabbit immunoglobulins (DAKO LSAB 2 Kit, Dako,

USA) for 10 min. The slides were rinsed and bathed in TBS, incubated with strepavidin peroxsidase (DAKO LSAB 2 Kit, Dako, USA) for 10 min, rinsed three times in TBS, exposed to diaminobenzidine (KPL Inc. USA) for 5 min, rinsed briefly with distilled water then counterstained with hematoxylin. Sections then were dehydrated in a graded series of alcohols and followed by xylene. They were then mounted and covered with a coverslip with a synthetic mounting media (DPX 8711) (Difco Lab. UK).

Counting and quantification

Counting of the CRF containing neurons were done using Leitz light microscope with a magnification of 40 × 10. The area of the paraventricular nucleus was defined and the number of CRF and βEP containing neurons were counted. Results were expressed as the number of neurons per PVN of the hypothalamus per rat, and expressed as mean ± standard error of mean. The area of the PVN was $2mm^2$ and all the positive neurons which the cytoplasm were stained brown were counted. Results were expressed as mean ± sem. Data were analysed by analysis of variance followed by student-t test using the PC stat computer program. Results are significant when $P<0.05$.

RESULTS

Dense populations of CRF containing neurons were located in the paraventricular nucleus (PVN) of the hypothalamus as shown by others.[1] The highest concentration in the brain of immunoreactive (ir) βEP was in the hypothalamus.[22] Fig. 1 shows the immunohistochemistry of CRF and βEP containing neurons at the hypothalamus of normal, adrenalectomized and DM treated rats stained with LSAB method. In rats treated with DM, the CRF containing neurons decreased markedly to 220 ± 7 neurons ($P<0.001$) compared to control (Fig. 2). Rats which were given DOC showed a significant ($P<0.05$) reduction in CRF containing neurons (598 ± 19 neurons) compared to normal rats. Rats that were given drinking water containing GCA showed a similar pattern as in intact rats treated with DOC (595 ± 14 neurons, $P<0.01$) compared to rats given tap water. The CRF containing neurons in the PVN of the hypothalamus of adrenalectomized rats increased significantly ($P<0.01$) from 792 ± 54 neurons to 1231 ± 87 neurons (Fig. 3). Adrenalectomized rats which were treated with DM had similar number of CRF containing neurons as in normal rats (763 ± 37 neurons). Adrenalectomized rats treated with DOC had a decreased number of CRF containing neurons (957 ± 28 neurons $P<0.05$) compared to adrenalectomized rats.

The number of βEP containing neurons in the PVN of the hypothalamus of ADX rats was 1079 ± 80 neurons (Fig. 4) which was not significantly different from normal rats (1151 ± 71 neurons). In rats treated with DM, DOC and GCA, the number of βEP containing neurons was not significantly different from that of normal rats (1120 ± 87, 1178 ± 61 and 1119 ± 67 neurons respectively). However the naloxone treated rats had significantly increased number of βEP containing neurons compared to the normal rats (1662 ± 52 neurons $P<0.001$).

DISCUSSION

In this study we confirmed that adrenalectomy caused an increase in CRF containing neurons in the PVN.[7-9] A 52% increase in hybridizable CRF mRNA in the whole hypothalamus 7 days after adrenalectomy has been described.[10] By using in situ hybridization, there was a more substantial increase (222%) of CRF mRNA in the PVN.[23] Northern blot analysis showed 60% increase in the whole hypothalamus and a 185% increase in the PVN by in situ hybridization after adrenalectomy.[24] On the other hand, adrenalectomy had no effect on the number of βEP containing neurons at the PVN of the hypothalamus. This finding is consistent with previous studies. Immunoreactive (ir) βEP levels in the hypothalamus after adrenalectomy were not significantly different between control and treated animals.[25] Dehydration and adrenalectomy caused a significant reduction of ir βEP in the pituitary gland but the ir βEP level in the hypothalamus was not altered.[26] Adrenalectomy increased anterior pituitary and plasma ir ACTH and ir βEP but did not alter hypothalamic and neurointermediate lobe (NIL) levels of either peptide.[21]

Treatment of intact rats with DM resulted in reduction of CRF containing neurons as shown by others. Glucocorticoids provide a potent inhibitory influence on CRF mRNA expression in the PVN.[10,23,24,27] In ADX rats treated with DM, the number of CRF containing neurons in the PVN were the same as in normal rats. The effects induced by adrenalectomy were abolished by glucocorticoid replacement.[10,11,24] However in intact rats that were given DM, we found no change in the number of βEP containing neurons

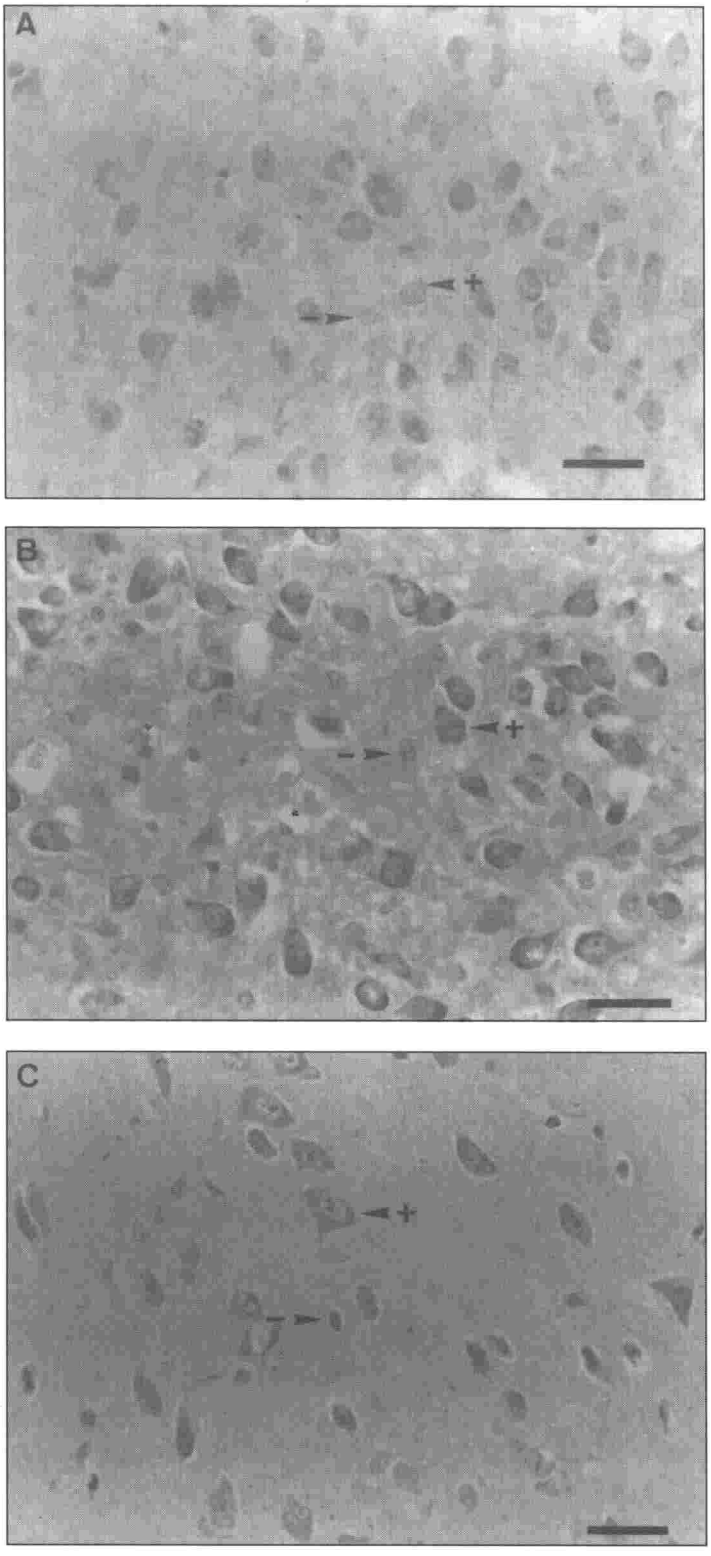

FIG. 1: CRF (corticotrophin releasing factor) containing neurons in rat PVN (paraventricular). Figure 1a: in normal, 1b: in adrenalectomized rats and Figure 1c: in dexamethasone treated rats. Arrow (+) demonstrating example of CRF containing neuron in which the cytoplasm are stained brown. Arrow (-) showing example of negative CRF containing neurons which the cytoplasm are not stained. (Scale bars: 20 mm).

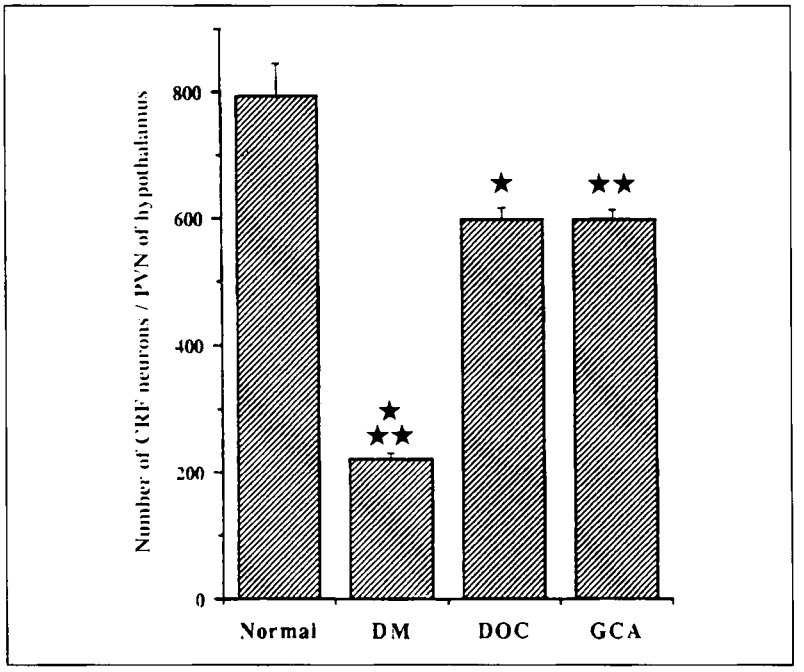

FIG. 2: Number of CRF (Corticotrophin releasing factor) containing neurons at the PVN (Paraventricular nucleus) of the hypothalamus in intact rats. DM: rats given dexamethasone 120 μg/kg intramuscularly daily for 7 days, DOC: rats given deoxycorticosterone 2.4 mg/kg intramuscularly daily for 7 days and GCA: rats given Glycyrrhizic acid 0.1 mg/ml orally for 9 weeks. ★ P<0.05 for DOC , ★★ P<0.01 for GCA and ★★★ P<0.001 for DM compared to control. Bars indicate SEM.

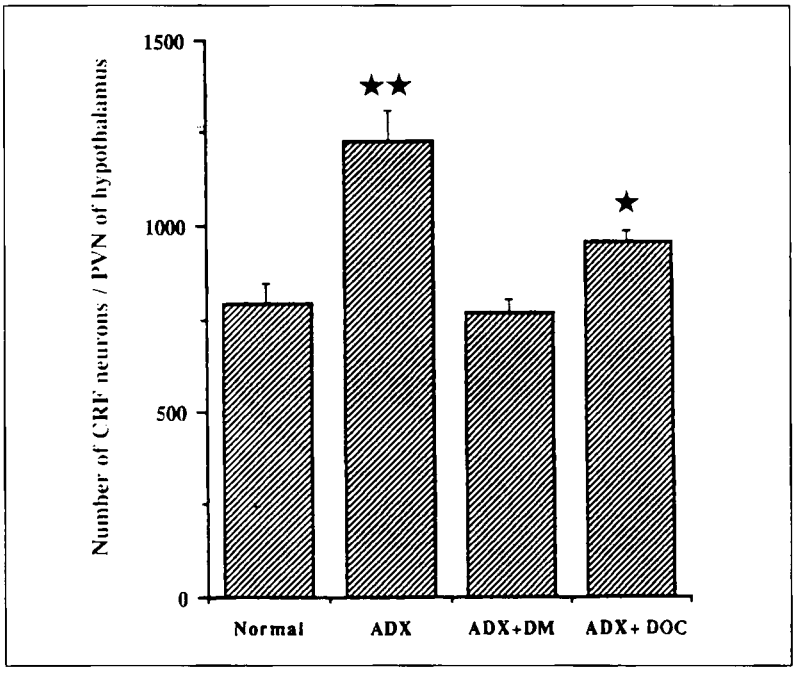

FIG. 3: Number of CRF (corticotrophin releasing factor) containing neurons at the PVN (paraventricular nucleus) of the hypothalamus of ADX (adrenalectomized rats). ADX+DM: adrenalectomized rats treated with dexamethasone 120 μg/kg intramuscularly daily for 7 days and ADX+DOC: adrenalectomized rats given deoxycorticosterone 2.4 mg/kg intramuscularly daily for 7 days. ★ P<0.05 for ADX+DOC and ★★ P<0.01 for ADX+DM compared to intact rat. Bars indicate SEM.

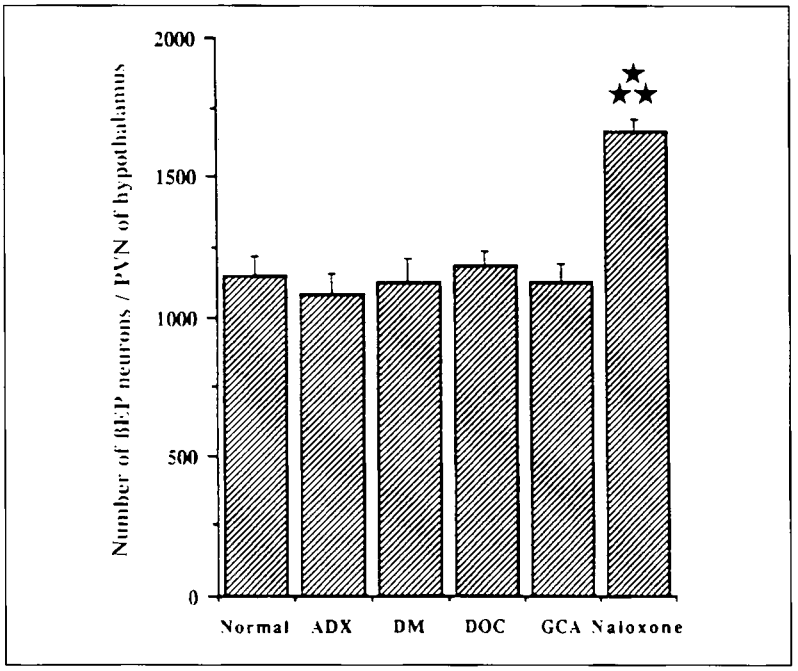

FIG. 4: Number of βEP (β-endorphin) containing neurons at the PVN (paraventricular nucleus) of the hypothalamus. ADX: adrenalectomized rats, DM: rats treated with dexamethasone, DOC: rats treated with deoxycorticosterone and GCA: rats treated with glycyrrhizic acid. Naloxone: rat given 0.32 μg/ 100g intramuscularly daily for 7 days. ADX, DM, DOC and GCA showed no significant differences compared to normal. ★★★ P<0.001 for rats treated with naloxone compared to normal. Bars indicate SEM.

(Fig. 4). This is consistent with the earlier findings by others. No significant differences were found in the level of ir βEP in the hypothalamus after administration of DM between control and treated rats.[25] Dexamethasone suppressed both plasma and anterior pituitary ir ACTH and ir βEP, with no change in hypothalamic or NIL levels of either peptide.[21]

In rats treated with mineralocorticoid DOC, the CRF containing neurons in the PVN decreased in number but not as much as the reduction in DM treated rats. These changes suggest that DOC also inhibited CRF containing neurons in the PVN but the effects were less compared to the effect by DM. The GCA treated rats showed similar results as in the DOC treated rats. Specific brain regions including the hippocampus and hypothalamus have functional 11HSD enzyme in vivo.[28] GCA inhibits the enzyme 11HSD resulting in accumulation of active steroid corticosterone which cause a reduction in CRF containing neurons in the PVN of the hypothalamus. Administration of glycyrrhetinic acid the active component of GCA, decreased CRF release into hypophysial portal blood in the presence of unchanged circulating glucocorticoid levels.[19] This suggest that the enzyme 11HSD regulates the effective corticosterone feedback signal to CRF neurons. 11HSD in the PVN may represent an important control point of corticosteroid feedback on CRF containing neurons.[19] GCA administration to rats in vivo resulted in inhibition of 11HSD activity and also a significant reduction in 11HSD mRNA level in mineralocorticoid and glucocorticoid target tissues.[29] We had earlier found that GCA treated rats and DOC treated rats demonstrated the same response to repeated exposure to ether vapour stress. Both block the stress induced hypotension and we proposed that these effects were due to inhibition of β-endorphins release.[20] However, the βEP containing neurons at the hypothalamus of DOC and GCA treated rats showed no significant differences compared to control rats. Deoxycorticosterone produced no significant changes in plasma, anterior pituitary or hypothalamic level of either peptide but significantly elevated NIL level of βEP.[21] Mineralocorticoids specifically elevated the NIL levels of both POMC and its immunoreactive

product, βEP.[30] Hypothalamic content of βEP does not appear to be affected by steroids.[31]

We administer Naloxone to the rats because it is an opioid antagonist and we wanted to see the antagonistic effect of Naloxone. Naloxone treated rats showed a significant increase in the number of βEP containing neurons. Naloxone caused a significant release of βEP from hypothalamic extract but not from pituitaries extract and these suggest that stimulation of release of endogenous opioid peptides by opiates occur at a suprapituitary level.[32] High doses of morphine was found to increase plasma ir βEP. This release of βEP into plasma was accompanied by a significant reduction of βEP content in the anterior lobe of the pituitary and the hypothalamus.[33] In our study, naloxone the opiate antagonist caused a significant elevation of βEP containing neurons at the PVN of the hypothalamus probably by inhibiting the release of βEP into plasma.

From this study we conclude that DOC and GCA have similar effects on CRF containing neurons at the PVN of the hypothalamus. Both caused inhibitory effects on the CRF containing neurons thus affecting the hypothalamic-pituitary-adrenal axis although via different mechanism. However, the number of βEP containing neurons at the hypothalamus are not affected by administration of glucocorticoid, mineralocorticoid or GCA implying that βEP from the hypothalamus is not responsible for the stress induced hypotension.

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REFERENCES

1. Bloom FE, Battenberg ELF, Rivier J, Vale W. Corticotropin-releasing factor (CRF) immunoreactive neurons and fibers in rat hypothalamus. Regul Peptides 1982; 4:43-8.
2. Cummings S, Elde R, Ells J, Lindall A. Corticotropin-releasing factor immunoreactivity is widely distributed within the central nervous system of the rat: an immunohistochemical study. J Neurosci 1985; 3:1355-68.
3. De Souza EB, Insel TR, Perrin MH, Rivier J, Vale WW, Kuhar MJ. Corticotropin-releasing factor receptors are widely distributed within the rat central nervous system: an autoradiographic study. J Neurosci 1985;5: 3189-203.
4. Merchanthaler I, Vigh S, Petrusz P, Schally AV. Immunocytochemical localization of corticotropin-releasing factor (CRF) in the rat brain. Am J Anat 1982; 165:385-96.
5. Olschowka JA, O'Donohue TL, Mueller GP, Jacobowitz DM. The distribution of corticotropin-releasing factor-like immunoreactive neurons in rat brain. Peptides 1982; 3:995-1015
6. Swanson LW, Sawchenko PE. Hypothalamic integration:organization of the Paraventricular and supraoptic nuclei.Ann Rev Neurosci 1983; 6:269-324.
7. Spinedi E, Giacomini M, Jacquier M-C, Gaillard RC. Changes in the hypothalamo-corticotrope axis after bilateral adrenalectomy: Evidence for a median eminence site of glucocorticoid action. Neuroendocrinology 1991; 53:160-70.
8. Bradbury MWB, Burden J, Hillhouse EW, Jones MT. Stimulation electrically and by acetylcholine of the rat hypothalamus in vitro. J Physiol 1974; 239:269-83.
9. Hillhouse EW, Jones MT (1976) Effects of bilateral adrenalectomy and corticosteroid therapy on the secretion of corticotropin releasing factor activity from the hypothalamus of the rat in vitro. J Endocrinol 1976; 71:21-30.
10. Jingami H, Matsukura S, Numa S, Imura H. Effects of adrenalectomy and dexamethasone administration on the level of preprocorticotropin-releasing factor messenger ribonucleic acid (mRNA) in the hypothalamus and adrenocorticotropin/β-lipotropin precursor mRNA in the pituitary in rats. Endocrinology 1985; 117:1314-20.
11. Keller-Wood M, Dallman MF. Corticosteroid inhibition of ACTH secretion. Endocrine Rev 1984; 5:1-24.
12. Levin N, Shinsako J, Dallman MF. Corticosterone acts on the brain to inhibit adrenalectomy-induced adrenocorticotropin secretion. Endocrinology 1988; 122:694-701.
13. Bloom F, Battenberg E, Rossier J, Ling N, Guillemin R. Neurons containing β-endorphin in rat brain exist separately from those containing enkephalin: Immunocytochemical studies. Proc Natl Acad Sci USA 1978; 75:1591-5.
14. Kalin NH, Risch SC, Cohen RM, Insel T, Murphy DL. Dexamethasone fails to suppress β-endorphin plasma concentrations in humans and rhesus monkeys.Science 1980;209:827-8.
15. Stewart PM, Wallace AM, Atherden SM, Shearing C, Edwards CRW. Mineralocorticoid activity of carbenoxolone: contrasting effects of carbenoxolone and liquorice on 11β-hydroxysteroid dehydrogenase activity in man. Clin Sci 1990; 78:49-54.
16. Moisan MP, Seckl JR, Edwards CRW. 11β-hydroxysteroid dehydrogenase bioactivity and messenger RNA expression in rat forebrain: localization in hypothalamus, hippocampus and cortex. Endocrinology 1990; 127:1450-5.
17. Ruszymah BHI, Nabishah BM, Aminuddin S, Khalid BAK. Effects of glycyrrhizic acid on right

atrial pressure and pulmonary vasculature in rats. Clin Exp Hypertens 1995; 17:575-91.
18. Krahenbuhl S, Hasler F, Krapf R. Analysis and pharmacokinetics of glycyrrhizic acid and glycyrrhetinic acid in humans and experimental animals. Steroids 1994; 59:121-6.
19. Seckl JR, Dow RC, Low SC, Edwards CRW, Fink G. The 11β-hydroxysteroid dehydrogenase inhibitor glycyrrhetinic acid affects corticosteroid feedback regulation of hypothalamic corticotrophin-releasing peptides in rats. J Endocrinol 1993; 136:471-7.
20. Ruszymah BHI, Nabishah BM, Aminuddin S, Khalid BAK. Mineralocorticoid and glycyrrhizic acid block stress induced hypotension in rats. Clin.Exp Pharmaco Physiol 1975; 22:35-9.
21. Lim AT, Khalid BAK, Clements J, Funder JW. Glucocorticoid and mineralocorticoid effects on adrenocorticotropin and β-endorphin in the adrenalectomized rat. J Clin Invest 1982; 69:1191-8.
22. Rossier J, Vargo TM, Minick S, Ling N, Bloom FE, Guillemin R. Regional dissociation of β-endorphin and enkephalin contents in rat brain and pituitary. Proc Natl Acad Sci USA 1977; 74:5162-5.
23. Young WS III, Mezey E, Siegel RE. Quantitative in situ hybridization histochemistry reveals increased levels of corticotropin-releasing factor mRNA after adrenalectomy in rats. Neurosci Lett 1986; 70:198-203.
24. Imaki T, Nahan JL, Rivier C, Sawchenko PE, Vale W. Differential regulation of corticotropin-releasing factor mRNA in rat brain regions by glucocorticoids and stress. J Neurosci 1991; 11:585-99.
25. Rossier J, French E, Gros C, Minick S, Guillemin R, Bloom FE. Adrenalectomy, dexamethasone or stress alters opioid peptides levels in rat anterior pituitary but not intermediate lobe or brain. Life Sci 1979; 25:2105-12.
26. Tseng L-F, O'Rourke MA, Li CH, Loh HH. Reduction of β-endorphin content in the rat pituitary after dehydration and adrenalectomy. Int J Peptide Protein Res 1979; 14:213-5.
27. Swanson LW, Simmons DM. Differential steroid hormone.and neural influences on peptide mRNA levels in CRH cells of the paraventricular nucleus: a hybridization histochemical study in the rat. J Comp Neurol 1989; 285:413-5.
28. Seckl JR, Kelly PAT, Sharkey J. Glycyrrhetinic acid, an inhibitor of 11β-hydroxysteroid dehydrogenase, alters local cerebral glucose utilization in vivo. J Steroid Biochem Mol Biol 1991; 39:777-9.
29. Whorwood CB, Sheppard MC, Stewart PM. Licorice inhibits 11β-hydroxysteroid dehydrogenase messenger ribonucleic acid levels and potentiates glucocorticoid hormone action. Endocrinology 1993;132:2287-92.
30. Khalid BAK, Lim AT, Fraillon DR, Funder JW. Mineralocorticoid and glucocorticoid effects on 31,000- and 29,000-Dalton proopiomelanocortin in rat anterior pituitary and neurointermediate lobe. J Clin Invest 1982;70:443-52.
31. Lim ATW, Funder JW. Stress-induced changes in plasma, pituitary and hypothalamic immunoreactive β-endorphin: effects of diurnal variation, adrenalectomy,corticosteroids and opiate agonists and antagonists. Neuroendocrinology 1983; 36:225-34.
32. Levin ER, Sharp B, Meyer NV, Carlson HE. Morphine and naloxone: effects on β-endorphin immunoreactivity in canine plasma and secretions from rat pituitaries. Endocrinology 1981;109:146-51.
33. Höllt V, Przewlocki R, Herz A. β-endorphin-like immunoreactivity in plasma, pituitaries and hypothalamus of rats following treatment with opiates. Life Sci 1978; 23: 1057-66.