COERULEAR ACTIVATION BY CRH AND ITS ROLE IN HYPERTENSION INDUCED BY PRENATAL MALNUTRITION IN THE RAT

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The effects of intracoerulear CRH and intraparaventricular prazosin on systolic pressure, diastolic pressure and heart rate were studied in prenatally malnourished hypertensive rats. At day 40 of life, (i) malnourished rats showed enhanced systolic pressure, heart rate, and plasma corticosterone; (ii) intracoerulear CRH increased systolic pressure and heart rate only in controls; (iii) intraparaventricular prazosin decreased systolic pressure and heart rate only in malnourished rats; (iv) in controls, prazosin did not prevent the stimulatory effect of CRH on the cardiovascular parameters; in malnourished rats, prazosin allowed CRH regain its stimulatory effects. Thus, coerulear activation by CRH would be involved in hypertension and tachycardia developed by prenatally malnourished animals.

Keywords: CRH, hypertension, locus coeruleus, paraventricular nucleus, prazosin, prenatal malnutrition

It has been reported that animals submitted to fetal growth retardation by *in utero* malnutrition can develop hypertension when adults (Langley-Evans et al., 1996a, b; Pérez et al., 2002). This postnatal hypertensive state is thought to be the result of physiological changes (fetal programming) in gene expression patterns that may affect neuroendocrine regulation and growth of cardiovascular and renal tissues during their most critical times of development (Barker, 1992; Seckl, 2001; Zicha & Kunes, 1999). Some hypertensive states are associated with hyperactivity of the hypothalamus-pituitary-adrenal (HPA) axis (al'Absi & Arnett, 2000; Bjorntorp et al., 2000; Grassi, 1998; Hashimoto et al., 1989). In this regard, small for gestational age children, which are in high risk to develop hypertension at adulthood (Barker, 1992), increased levels of corticotropin releasing hormone (CRH), adrenocorticotropic hormone, and glucocorticoid concentrations have been documented (Goland et al., 1993). It seems that enhanced HPA activity and hypertension are both linked to increased hypothalamic CRH activity, because increased CRH mRNA expression in the paraventricular nucleus (PVN) of spontaneously hypertensive rats (Krukoff et al., 1999) and in the hypothalamus of prenatal malnutrition-induced hypertensive rats (Pérez et al., 2004) have been reported. Furthermore, increases in CRH mRNA expression and in the number of cells expressing CRH have been observed in the PVN of humans with primary hypertension (Goncharuk

et al., 2002). Together with enhanced CRH mRNA expression, prenatally malnourished rats also show increased central noradrenergic activity during postnatal life, as revealed by increased synthesis (Marichich et al., 1979; Soto-Moyano et al., 1998a) and release (Soto-Moyano et al., 1998a, b) of noradrenaline in the brain of these animals, and by increased spontaneous neuronal activity in the locus coeruleus (LC) of perinatally malnourished rats (Nasif et al., 2001). CRHergic an noradrenergic hyperactivity are likely to coexist in the brain of these malnourished animals, because reciprocal excitatory connections there exist between neurons of the PVN and LC (Dunn et al., 2004).

As a whole, these data point to an important role for PVN and LC in the pathogenesis of hypertension that prenatally malnourished rats develop during their postnatal life, but conclusive data supporting this contention are still lacking. To address this question, the effects of intracoerulear CRHergic agonists and intraparaventricular adrenoceptor antagonists on systolic pressure, diastolic pressure, and heart rate were studied in both prenatal malnutrition-induced hypertensive and control normotensive young rats. Additionally, basal levels of plasma corticosterone were measured in blood samples taken from normal and malnourished rats of 40 days of age, in order to confirm hyperactivity of the HPA axis in the malnourished groups. As previously reported, undernutrition of pregnant rats resulted in increased plasma corticosterone in adult offspring (Sohlstrom et al., 2000).

METHODS

Animals

The experimental protocol and animal management were in accordance with the NIH Guide for the Care and Use of Laboratory Animals (National Research Council, 1985) and were approved by the Committee for the Ethical Use of Experimental Animals, INTA, University of Chile. The experiments were carried out on male and female Wistar rats (INTA, Santiago, Chile), born from mothers subjected during pregnancy to one of the following nutritional conditions: (i) normal pregnant rats, with free access to a 21% protein standard laboratory diet (Champion, Santiago, Chile); (ii) malnourished pregnant rats, with restricted access (10 g daily) to the standard laboratory diet throughout pregnancy; this amount of food is about 40% of that consumed by normal pregnant rats (Soto-Moyano et al., 1998a). At birth, prenatally malnourished pups were fostered to well-nourished dams giving birth on that day, whereas

pups born from well-nourished dams were nursed by their own mothers. During the lactation period all litters were adjusted to eight pups per dam, and all dams continued to receive the standard laboratory diet ad libitum. After weaning at 22 days of age, all pups were housed eight per cage and fed on the standard laboratory diet. The body weight of pregnant mothers and the body weight of pups were measured daily.

Arterial Pressure and Heart Rate

At 40 days of age, rats were anesthetized with 1.2 g/kg urethane and placed in a Narishige stereotaxic apparatus for rats. The horizontal zero plane of the stereotaxic apparatus was tangent to the upper incisor bar and 5 mm above the interaural line. Body temperature was maintained at 37 to 38°C. The skull was exposed, and a 3.0-mm diameter hole, centered at 1.0 mm lateral to the midline and 7.6 mm caudal to the bregma point, was drilled over the right occipital pole for approaching the LC according to the stereotaxic atlas of the rat brain by Pellegrino et al. (1979). A second 3.0-mm diameter hole, centered at 0.5 mm lateral to the midline and 0.6 mm rostral to the bregma point, was drilled in the right parietal bone for approaching the PVN. The dura was then carefully removed using fine forceps and iridectomy scissors. Two micropipettes of 20 mm tip were advanced to the LC and PVN, respectively, for microinjecting either 0.5 μg of CRH into the LC, or 1.2 μg of the α_1 adrenoceptor antagonist prazosin into the PVN. Stereotaxic coordinates were taken from Pellegrino et al. (1979): for the LC coordinates were A, -1.6; L, 1.0; V, -2.8, in mm, and for the PVN coordinates were A, 6.4; L, 0.5; V, -1.6, in mm. Drugs were dissolved in artificial cerebrospinal fluid (CSF) and microinjections were performed in a 0.05 μl volume with a microinfusion pump; injection of the entire volume required 1–2 min. Both systolic and diastolic arterial blood pressures as well as heart rate were measured from the rat's tail by means of a noninvasive blood pressure system (XBP 1000 Kent Scientific apparatus, Torrington, CT, USA), before and 2, 5, 10, 15, 20, 25, and 30 min after drug microinjection of CRH into the LC. Afterward, the animals were killed by intracardiac perfusion of 10% formalin solution, and the brains were removed and processed histologically for verification of electrode placement in the respective nuclei.

Plasma Corticosterone

At 40 days of age, additional normal and malnourished rats were sacrificed by decapitation. Blood samples of about 0.5 ml each were collected and centrifuged at 3000 rpm during 10 min at 4°C. Plasma corticosterone was measured using

a radioimmunoassay (RIA) based on ^{125}I-labelled rat corticosterone that was performed according to the manufacturer's instructions (Diagnostic Products Corporation, Los Angeles, CA, USA). This Coat-a-Count rat corticosterone procedure is a solid-phase RIA in which rat ^{125}I-corticosterone competes for a fixed time (120 min) with corticosterone in the sample for antibody sites. The antibody is coated on the wall of a polypropylene tube. Decanting the supernatant is sufficient to terminate the competition and to isolate the antibody-bound fraction of the radiolabelled corticosterone. Counting the tube in a gamma counter (Riastar Packard, CT, USA) then yields a number that converts by way of a calibration curve to a measure of the corticosterone present in the sample. Intra and interassay coefficients of variation were approximately 5%.

Data Analyses

To determine the changes induced by the malnutrition regimen during pregnancy on body weight of pregnant mothers, as well as on body weight, brain weight, plasma corticosterone, arterial blood pressure, and heart rate of pups, values obtained from malnourished animals were expressed as means ± SD and compared to the corresponding values found in normal animals using unpaired two-tailed Student's *t*-test.

To assess changes induced by microinjection of drugs into LC and PVN on cardiovascular parameters, the systolic and diastolic arterial blood pressures and the heart rate were recorded. The time-course of effects of the drugs employed (intragroup comparisons) were analyzed using one-way ANOVA followed by the Dunnett multiple comparisons test (GraphPad Instat 3.00, GraphPad Software Inc., San Diego, CA, USA). To appreciate the global effect of drugs or CSF over the total period of testing in both normal and malnourished animals, the area under the curves (AUC) was determined. AUC was calculated as the integral from 0 to 30 min (period of testing) using the Origin 6.0 software, and AUC change was defined as AUC under drug minus AUC under CSF and plotted as bar graph. The effects of the drug treatment and the nutritional condition on AUC change (intergroup comparisons) were analyzed using two-way ANOVA followed by the Bonferroni *post hoc* test (GraphPad Prism 3.00, GraphPad Software Inc., San Diego, CA, USA).

RESULTS

Table 1 shows that, at day 40 of age, body ($p < .01$) and brain ($p < .001$) weights of malnourished rats were significantly decreased, whereas significant increases were observed in plasma corticosterone ($p < .01$), systolic pressure

Table 1. Body weight, brain weight, plasma corticosterone, systolic pressure, diastolic pressure, and heart rate of normal and malnourished rats at day 40 of age

	Normal group	Malnourished group
Body weight (g)	151.4 ± 17.4 (36)	139.4 ± 15.1* (36)
Brain weight (mg)	1368.2 ± 30.6 (36)	1298.4 ± 28.4** (36)
Corticosterone (ng/ml)	228.8 ± 80.4 (10)	325.7 ± 92.5* (10)
Systolic pressure (mm Hg)	128.6 ± 12.5 (18)	142.8 ± 16.3* (18)
Diastolic pressure (mm Hg)	78.2 ± 7.2 (18)	83.1 ± 8.5 (18)
Heart rate (beats/min)	353.5 ± 77.0 (18)	430.1 ± 88.4* (18)

Values are means ± SD. Parentheses enclose number of rats. Significance of difference (Student's t-test) between normal and malnourished rats: $^{*}p < .01$; $^{**}p < .001$.

($p < .01$) and heart rate ($p < .01$) in these animals. No statistical significant difference was observed in diastolic pressure of the malnourished group when compared to the value from the normal one (Table 1).

Figure 1A shows that at $t = 0$ min the systolic arterial pressure in the malnourished group was significantly higher than that of the normal group ($p < .01$). Microinjection of 0.5 μg of CRH into the LC increased the systolic pressure recorded from normal rats ($p < .05$, at 5 and 10 min after CRH microinjection), whereas systolic pressure of malnourished animals was not significantly modified. Figure 1 also shows that microinjection of 1.2 μg prazosin into the PVN induced a significant decrease of the systolic pressure in the malnourished group ($p < .001$), which reaches similar scores to those observed in the normal group. In contrast, prazosin microinjection into the PVN did not modify the systolic pressure in normal rats. CRH administration within the LC 10 min after prazosin microinjection into the PVN induced significant increases of systolic pressure of both normal ($p < .05$, 10 min after CRH microinjection) and malnourished ($p < .05$, 25 and 30 min after CRH microinjection) rats. Thus, in malnourished animals, systolic pressure could only be increased by CRH after normalizing the blood pressure with prazosin. Figure 1B shows the global effect of the CRH microinjection over the total period of testing (30 min). It can be observed that CRH microinjection into the LC failed to induce systolic pressure increases only in malnourished animals without prazosin pretreatment ($p < .05$). In contrast, in prazosin pretreated malnourished animals CRH microinjection into the LC produced systolic pressure enhancements, indicating that preinfusion of prazosin into the PVN modified the effect of CRH in systolic blood pressure of malnourished animals (significant interaction prazosin × nutritional condition in the two-way ANOVA, $p < .05$).

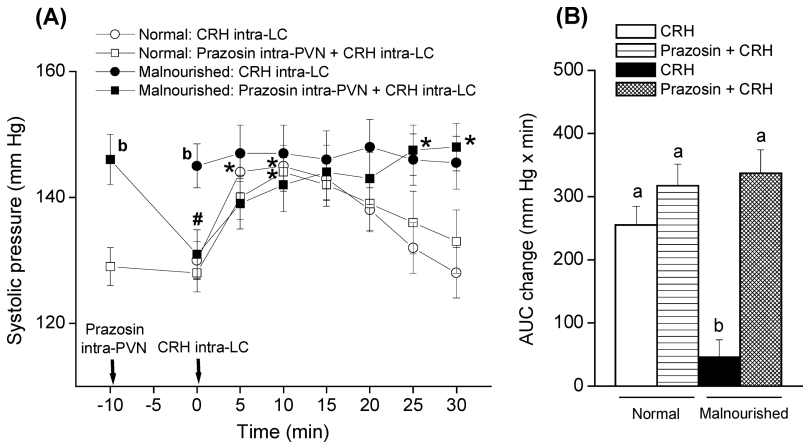

Figure 1. (A): Time-course of changes in systolic arterial pressure of normal and prenatally malnourished rats after intra-LC microinjection of CRH (0.5 μg/0.05 μl, right arrow), with or without prazosin pretreatment (intra-PVN microinjection, 1.2 μg/0.5 μl, left arrow). Values are means $\pm$ SEM, $n = 8$ rats in each group. The effect of the intra-LC microinjection of CRH over the time-course (intragroup comparison) was analyzed using one-way ANOVA followed by the Dunnett post hoc test. For normal rats receiving only CRH microinjection, p ANOVA$<$ .0001, $F = 6.676$; for normal rats receiving CRH preceded by prazosin, p ANOVA$<$ .0002, $F = 4.896$; for malnourished rats receiving only CRH, p ANOVA $=$ .9991, $F =$ .0791; for malnourished rats receiving CRH preceded by prazosin, p ANOVA$<$ .0005, $F = 4.453$. In these series, the probability level for comparison of systolic pressures obtained after CRH microinjection (at times 2 to 30 min) with the corresponding systolic pressure measured immediately before CRH microinjection (at time 0 min) was $^{*}p < .05$ (Dunnett multiple comparisons test). The effect of the prazosin microinjection into the PVN on systolic pressure of normal and prenatally malnourished rats was analyzed by comparing pre-prazosin values (at time -10 min) with post-prazosin values (at time 0 min) using two-tailed Student's t-test: $^{\#}p < .001$. The effect of the nutritional condition was analyzed by comparing corresponding systolic pressures from the normal groups to that from the malnourished groups (at times -10 and 0 min) using two-tailed Student's t-test: $^{b}p < .01$. **(B)**: The area under curves (AUC) was calculated as the integral from 0 to 30 min (Microcal Origin 5.0 software); AUC change was defined as AUC under drug (data from graph A) minus AUC under CSF (data not shown) and plotted as bar graph. The effect of prazosin pretreatment as well as the effect of the nutritional condition on AUC change (intergroup comparisons) was analyzed using two-way ANOVA followed by the Bonferroni post hoc test. The prazosin variable showed a p ANOVA$<$ .05, $F = 3.03$, and the nutritional condition showed a p ANOVA$<$ .05, $F = 6.60$; the interaction showed a p ANOVA$<$ 0.05, $F = 4.71$. Intergroup comparisons (Bonferroni multiple comparisons test) indicated that means without common superscripts are significantly different ($p < .05$).

Figure 2A shows that the diastolic arterial pressure in malnourished rats did not significantly differ to that of normal animals. Microinjection of 0.5 μg of CRH into the LC did not elicite significant changes in diastolic pressure either in normal or in malnourished rats. Figure 2A also shows that pre-infusion

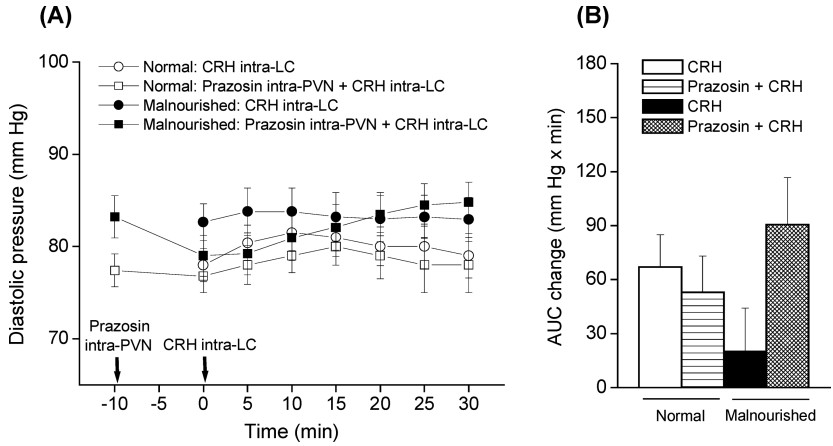

Figure 2. **(A)**: Time-course of changes in diastolic arterial pressures of normal and prenatally malnourished rats after intra-LC microinjection of CRH (0.05 μg/0.05 μl, right arrow), with or without prazosin pretreatment (intra-PVN microinjection, 1.2 μg/0.5 μl, left arrow). Values are means $\pm$ SEM, $n = 8$ rats in each group. The effect of the intra-LC microinjection of CRH over the time-course (intragroup comparison) was analyzed using one-way ANOVA followed by the Dunnett post hoc test. For normal rats receiving only CRH microinjection, p ANOVA $= .9264$, $F = .3146$; for normal rats receiving CRH preceded by prazosin, p ANOVA $= .9791$, $F = .1871$; for manourished rats receiving only CRH, p ANOVA $= .9990$, $F = .0310$; for malnourished rats receiving CRH preceded by prazosin, p ANOVA $= .3980$, $F = 1.062$. In these series, the probability level for comparison of diastolic pressures obtained after CRH microinjection (at times 2 to 30 min) with the corresponding diastolic pressure measured before CRH microinjection (at time 0 min) was $^{*}p < .05$ (Dunnett multiple comparisons test). The effect of the prazosin microinjection into the PVN on diastolic pressures of normal and prenatally malnourished rats was analyzed by comparing pre-prazosin values (at time -10 min) with post-prazosin values (at time 0 min) using two-tailed Student's t-test: no significant differences were found. The effect of the nutritional condition was analyzed by comparing corresponding diastolic pressures from the normal groups to that from the malnourished groups (at times -10 and 0 min) using two-tailed Student's t-test: no significant differences were found. **(B)**: AUC changes and two-way ANOVA statistics were determined as in Fig. 1B. The prazosin variable showed a p ANOVA $= .8410$, $F = .04$, and the nutritional condition showed a p ANOVA $< .2150$, $F = 1.61$; the interaction showed a p ANOVA $= .0680$, $F = 3.60$. Intergroup comparisons (Bonferroni multiple comparisons test) indicated no significant differences between groups.

of 1.2 μg prazosin into the PVN did not produce effects by its own on diastolic pressure recorded from normal and malnourished animals, nor modified the lack of effect of CRH on this parameter. Figure 2B shows the global effect of the CRH microinjection over the total recording period of diastolic pressure. It can be observed that CRH microinjection into the LC failed to induce significant

changes in diastolic pressure in normal and malnourished animals. Similarly, CRH microinjection into the LC of prazosin pretreated rats did not produce any significant change in diastolic pressure.

Figure 3A shows that at $t = 0$ min the heart rate in the malnourished group was significantly higher than that of the normal group ($p < .01$). Microinjection of 0.5 μg of CRH into the LC increased the heart rate recorded

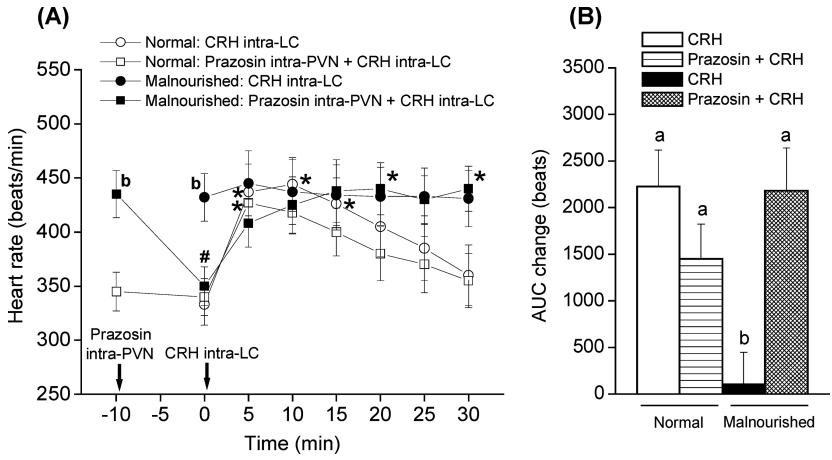

Figure 3. **(A)**: Time-course of changes in heart rate of normal and prenatally malnourished rats after intra-LC microinjection of CRH (0.5 μg/0.05 μl, right arrow), with or without prazosin pretreatment (intra-PVN microinjection, 1.2 μg/0.05 μl, left arrow). Values are means $\pm$ SEM, $n = 8$ rats in each group. The effect of the intra-LC microinjection of CRH over the time-course (intragroup comparison) was analyzed using one-way ANOVA followed by the Dunnett post hoc test. For normal rats receiving only CRH microinjection, p ANOVA $< .0312$, $F = 2.555$; for normal rats receiving CRH preceded by prazosin, p ANOVA $< .0439$, $F = 2.452$; for malnourished rats receiving only CRH, p ANOVA $= .9990$, $F = .0254$; for malnourished rats receiving CRH preceded by prazosin, p ANOVA $= .0477$, $F = 2.408$. In these series, the probability level for comparison of heart rates obtained after CRH microinjection (at times 2 to 30 min) with the corresponding heart rate measured before CRH microinjection (at time 0 min) was $^{*}p < .05$ (Dunnett multiple comparisons test). The effect of the prazosin microinjection into the PVN on heart rate of normal and prenatally malnourished rats was analyzed by comparing pre-prazosin values (at time -10 min) with post-prazosin values (at time 0 min) using two-tailed Student's t-test: $^{\#}p < .001$. The effect of the nutritional condition was analyzed by comparing corresponding heart rates from the normal groups to that from the malnourished groups (at times –10 and 0 min) using two-tailed Student's t-test: $^{b}p < .01$. **(B)**: AUC changes and two-way ANOVA statistics were determined as in Fig. 1B. The prazosin variable showed a p ANOVA $= .1023$, $F = 2.85$, and the nutritional condition showed a p ANOVA $< .01$, $F = 8.68$; the interaction showed a p ANOVA $< .0004$, $F = 16.50$. Intergroup comparisons (Bonferroni multiple comparisons test) indicated that means without common superscripts are significantly different ($p < .001$).

from normal rats ($p < .05$, at 5, 10, and 15 min after CRH microinjection), whereas heart rate of malnourished animals was not significantly modified. Figure 3A also shows that microinjection of 1.2 μg prazosin into the PVN induced a significant decrease of the heart rate in the malnourished group ($p < .001$), which reaches similar frequency to that observed in the normal group. In contrast, prazosin microinjection into the PVN did not modify the heart rate in normal rats. CRH administration within the LC 10 min after prazosin microinjection into the PVN induced significant increases of heart rate in both normal ($p < .05$, 5 min after CRH microinjection) and malnourished ($p < .05$, 20 and 30 min after CRH microinjection) rats. Thus, in malnourished animals, heart rate could only be enhanced by CRH after normalizing the tachycardia effect with prazosin. Figure 3B shows the global effect of the CRH microinjection over the total period of heart rate recording (30 min). It can be observed that CRH microinfusion into the LC failed to induce tachycardia only in malnourished animals without prazosin pretreatment. In contrast, in prazosin pretreated malnourished animals CRH microinjection into the LC increased heart rate, indicating that pre-infusion of prazosin into the PVN modified the chronotropic effect of CRH in the malnourished group (significant interaction prazosin $\times$ nutritional condition in the two-way ANOVA, $p < .0004$).

DISCUSSION

Brain weight measurements performed in rats of 40 days of age revealed a significant brain weight deficit in the malnourished group. As reported previously, prenatal malnutrition could result in long-lasting brain weight deficits through a mechanism involving losses of neurons, glia and myelin, and impaired dendritic differentiation, among other factors (Morgane et al., 1993). At 40 days of postnatal life, plasma corticosterone was increased in malnourished rats. This is in line with previous reports showing that maternal food restriction during gestation (Sohlstrom et al., 2000) or maternal food restriction during gestation and lactation (Sebaai et al,. 2004) results in high plasma levels of corticosterone in adult offspring, suggesting that maternal malnutrition programming for permanent alterations in glucocorticoid secretion in the progeny. In agreement with animals studies, low human birth weight, as an aproximation for undernutrition *in utero*, is associated with increased urinary glucocorticoid secretion in children (Clark et al., 1996) and increased plasma levels of glucorcorticoids in adult men (Walker et al., 1998).

At 40 days of age, significant increases of both systolic pressure and heart rate were observed in malnourished rats. As mentioned earlier, hypertension can

develop in rat progeny by submitting normotensive rat mothers to undernutrition during pregnancy (Langley-Evans et al., 1996a, b; Pérez et al., 2002). As discussed elsewhere (for review see Seckl, 2001), this is probably caused by a series of sequential events induced by prenatal malnutrition including: (i) decreased activity of placental 11β-hydroxysteroid dehydrogenase type 2, which catalyses the rapid metabolism of cortisol and corticosterone to inert steroids, resulting in increased exposure of the fetal brain to glucocorticoids of maternal origin (Langley-Evans et al., 1996a, b); (ii) this leads to decreased glucocorticoid receptor expression during fetal life in regions concerned with the regulation of the HPA axis, such as the hypothalamus (Bertram et al., 2001), the pituitary (Hawkins et al., 2001) and hippocampus (Lesage et al., 2001), a structure with the highest density of corticosteroid binding sites in the brain and an important site of feedback control upon the HPA axis (Levitt et al., 1996; Welberg et al., 2001); (iii) the reduced negative feedback control by glucocorticoids results in higher expression of CRH during postnatal development (Pérez et al., 2004), a peptide serving as a positive signal to the HPA axis (causing increased plasma levels of corticosterone) but also to extra-hypothalamic brain regions such as the LC (Dunn et al., 2004); (iv) both factors, enhanced plasma corticosterone levels and increased neuronal activity of LC neurons, may account for the hypertensive state showing prenatally malnourished rats, the first by acting directly on vascular glucocorticoid receptors (Yang & Zhang, 2004) and the later by activating the sympathoadrenomedullary system (Drolet & Gauthier, 1985, 1987) and/or by depressing the baroreceptor reflex (Chan et al., 1992).

Microinjection of CRH into the LC of normal rats produced slight but significant elevations of systolic pressure and heart rate, which are in agreement with previous reports showing increased arterial blood pressure and/or heart rate after i. c.v. (Brown et al., 1988) and intra-LC (Ku et al., 1998) administration of CRH. Intracerebral CRH seems to increase blood pressure and heart rate by activating the sympathetic-adrenomedullary system since, on the one hand, centrally administered CRH results in both activation of adrenal sympathetic efferent nerve activity (Kurosawa et al., 1986) and increases in plasma noradrenaline and adrenaline (Nijsen et al., 2000) and, on the other hand, sympathetic blockade prevents CRH-induced tachycardia (Nijsen et al., 2000). The fact that prazosin microinjected into the PVN ten minutes before CRH microinfusion into the LC of normal animals did not prevent the increases in blood pressure and heart rate, suggests that the LC to PVN connection seems to be not essential for eliciting the cardiovascular effects observed. In other words, it seems likely that the sympathetic-adrenomedullary

system is stimulated via LC activation, but not via PVN activation. In the brain CRH can stimulate the G_s-adenylyl cyclase system (Grammatopoulos & Chrousos, 2002), a pathway that has been reported to induce neuronal excitation in the LC (Nestler et al., 1999). Some studies have reported ineffectiveness of CRH to modify the blood pressure after intracerebroventricular (Schulz et al., 1994) and intracoerulear (Curtis et al., 1997) administration in the rat. In this respect, it has been argued that centrally administered CRH is unable to increase blood pressure in anesthetized rats (Schulz et al., 1994). Nevertheless, recent data showed that LC neurons are similarly activated by CRH in anesthetized and unanesthetized rats, as revealed by chronoamperometric measurement of noradrenaline release in target brain sites for LC innervation (Dunn et al., 2004). In the present study CRH administered into the LC of urethane anesthetized rats gave rise to a slight but significant enhancement of systolic pressure, similar to that reported by Ku et al. (1998).

In contrast to the effects induced in normal rats, microinjection of CRH into the LC of hypertensive prenatally malnourished rats did not produce significant changes either in blood pressure or in heart rate. Similar results have previously been found by others in spontaneously hypertensive rats receiving CRH intracerebroventricularly (Brown et al., 1988). Ineffectiveness of CRH intra-LC to induce hypertension and tachycardia reported herein cannot be consequence of down-regulation of CRH receptors in the brain of hypertensive prenatally malnourished rats, because prazosin intra-PVN decreased systolic pressure and heart rate and concomitantly restored the ability of CRH to produce cardiovascular stimulation. It seems apparent, therefore, that excitatory effects of CRH on cardiovascular activity could be exerted only in normotensive animals, whereas in malnutrition-induced hypertension, CRH intra-LC was unable to elicit cardiovascular effects beyond the limits imposed by an already hyperactive system.

It seems worthy to point out that increases in hypothalamic CRH mRNA expression (Pérez et al., 2004) and in central noradrenergic activity (Soto-Moyano et al., 1998a) reported in prenatally malnourished rats is coherent with increased tonic activity in the PVN-LC feed-forward loop reported elsewhere (Dunn et al., 2004), giving a molecular support to such a notion. In this context, altered number of α and β adrenoceptors has been already observed in total brain (Keller et al., 1982) and neocortex (Seidler el al., 1990; Soto-Moyano et al., 2005) of perinatally undernourished rats, together with increased noradrenaline turnover (Marichich et al., 1979) and release (Soto-Moyano et al., 1998a, b). Further studies on adaptive changes occurring in brainstem adrenergic and hypothalamic CRHergic systems would be helpful

for a better understanding of the hypothalamic-coerulear mechanisms by which maternal malnutrition leads to fetal programming of hypertension at postnatal ages. Finally, the present data provide new light on the origin of prenatal malnutrition-induced hypertension in the rat, which is complementary to other explanations involving reduced nephron number in the kidney (Dodic et al., 2002) and increased vascular glucocorticoid receptor number (Langley-Evans et al., 1996c; Yang & Zhang, 2004), as part of the mechanisms programming hypertension in adult life.

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