Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 725–729, January 1989
Neurobiology

Angiotensin II receptors in paraventricular nucleus, subfornical organ, and pituitary gland of hypophysectomized, adrenalectomized, and vasopressin-deficient rats

(receptor autoradiography/corticotropin-releasing hormone/adrenocorticotropin/blood pressure regulation/Brattleboro rats)

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Communicated by Irvine H. Page, October 18, 1988 (received for review August 10, 1988)

ABSTRACT **Angiotensin II has been implicated in the regulation of adrenocorticotropin and vasopressin secretion. Angiotensin II may influence the secretion of these hormones either directly at the pituitary gland or by increasing corticotropin-releasing hormone or vasopressin release from cells that are located in the paraventricular hypothalamic nucleus. Pituitary hormone release may also be influenced by circulating angiotensin II through receptors outside the blood–brain barrier in the subfornical organ. We have used alterations in angiotensin II receptors in hypophysectomized, adrenalectomized, and vasopressin-deficient Brattleboro rats as indicators of the activity of angiotensin II in the regulation of adrenocorticotropin and vasopressin secretion. Angiotensin receptor number in the paraventricular nucleus and the subfornical organ, but not in the anterior pituitary gland, was significantly decreased by adrenalectomy, and this effect was reversed by corticoids. Vasopressin deficiency decreased angiotensin receptors in the subfornical organ and increased them in the anterior pituitary gland but did not affect angiotensin II binding in either magnocellular or parvocellular subnucleus of the paraventricular nucleus. Our results suggest that angiotensin II may have a corticoid-dependent role in the regulation of corticotropin-releasing hormone secretion, which could be important in the adaptation to elevated corticosterone secretion in stress.**

Angiotensin II is a physiologically active product of the renin–angiotensin system and has an important role in hemodynamic regulation (1). All components of the renin–angiotensin system have been found in the central nervous system (2). Brain angiotensin II seems to work in concert with the peripheral renin–angiotensin system to regulate blood pressure and fluid homeostasis (2). Both in the periphery and in the central nervous system angiotensin II acts through specific membrane receptors (3–5). In brain, angiotensin receptors show highly discrete localization in only few brain areas (4, 5). Angiotensin receptors in circumventricular organs, such as the subfornical organ, are accessible to circulating as well as central peptides, whereas those in other brain areas are inside the blood–brain barrier and can thus only be stimulated by endogenous brain angiotensin II (6).

Angiotensin II also plays a role in the regulation of adrenocorticotropin (ACTH) and vasopressin secretion from the pituitary gland (7–10). Vasopressin release is increased by central (10) and perhaps also by peripheral (11) administration of angiotensin II. Peripheral angiotensin II administration stimulates ACTH release from the anterior pituitary gland (12), but this effect may be mediated through the brain angiotensin II system: circulating angiotensin II may bind to receptors in circumventricular organs and stimulate the brain angiotensin II system, which leads to increased ACTH release probably through the stimulation of corticotropin-releasing hormone (CRH) secretion from the hypothalamus (9, 12).

The paraventricular nucleus is one of the key hypothalamic nuclei in the neuroendocrine regulation (13). It is divided into distinct, biochemically specific subnuclei (13). Neurons in the magnocellular subdivision, together with those in the supraoptic nucleus, produce vasopressin and oxytocin and project to the posterior pituitary gland, from where the hormones are released to the circulation (13). In the parvocellular subnucleus, neurons produce the releasing factors CRH, thyrotropin-releasing hormone, and somatostatin, which are released at the median eminence to control hormone secretion from the anterior pituitary gland (13). The subfornical organ sends a dense, angiotensin II-containing projection to the paraventricular nucleus (14), and angiotensin II-binding sites are concentrated along this pathway (15). It seems likely, therefore, that subfornical organ–paraventricular nucleus projection is at least one source for angiotensin II that binds to angiotensin receptors in the paraventricular nucleus and that this connection mediates the effects of circulating angiotensin II in the control of pituitary function (9, 12).

Angiotensin receptors are increased after repeated stress in the paraventricular nucleus and subfornical organ (16) and after dehydration in the subfornical organ (17). Increased concentration of circulating angiotensin II might upregulate its own receptors in stress and dehydration by a mechanism similar to that described for peripheral angiotensin receptors (18). However, adrenal corticoids and vasopressin are also increased after repeated stress and dehydration (19, 20). Mineralocorticoids have been reported to increase the number of central angiotensin receptors (21, 22); dexamethasone decreases angiotensin receptors in kidney (23) and increases brain angiotensinogen gene expression (24).

We have investigated the effects of adrenal steroids and vasopressin on angiotensin receptors in the subfornical organ, the paraventricular nucleus, and the anterior pituitary gland using hypophysectomized, adrenalectomized, and vasopressin-deficient Brattleboro rats. Our results demonstrate that the density of angiotensin receptors in the paraventricular nucleus and subfornical organ, but not in the anterior pituitary gland, can be regulated by corticoids.

MATERIALS AND METHODS

Hypophysectomized, adrenalectomized, and respective sham-operated male Wistar rats (8 weeks old) were pur-

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Abbreviations: ACTH, adrenocorticotropin; CRH, corticotropin-releasing hormone; DOCA, deoxycorticosterone acetate.

chased from Charles River Breeding Laboratories. Hypophysectomized rats were given 5% glucose to drink and were killed 7 days after the surgery. Adrenalectomized rats had access to 0.9% NaCl in drinking water, and they were killed 11 days after the surgery. Corticosterone replacement was given to eight adrenalectomized rats by adding corticosterone (Sigma) at 100 mg/liter for 1 week into drinking water; this administration route maintains almost normal circadian variation in plasma corticosterone levels (25). To selectively replace mineralocorticoid deficiency, deoxycorticosterone acetate (DOCA; Sigma) at 12.5 mg/kg was injected s.c. to eight rats twice weekly for 1 week. A preliminary experiment did not indicate significant differences in angiotensin II binding between vehicle-injected adrenalectomized rats and untreated adrenalectomized rats.

Male, 8-week-old homozygous and heterozygous Brattleboro rats and age-matched normal Long–Evans rats were obtained from Blue Spruce Farm and kept in individual cages. Urine output was monitored for 1 week, and homozygous Brattleboro rats that failed to urinate more than 50 ml/day during at least 2 consecutive days were excluded from the study.

All animals were killed by decapitation between 0930 hr and 1100 hr. Brains and pituitary glands were rapidly dissected out and immediately frozen in isopentane on dry ice. The completeness of hypophysectomies and adrenalectomies was confirmed by visual inspection. Trunk blood was collected for hormone assays, and thymuses of adrenalectomized rats were removed. Weights of thymuses were 401 ± 29 mg in sham-operated rats, 609 ± 37 mg in adrenalectomized rats ($P < 0.01$), 133 ± 21 mg in corticosterone-treated rats ($P < 0.001$), and 519 ± 54 mg in DOCA-treated rats, indicating that the corticosterone replacement therapy was successful (25). Hypophysectomized and adrenalectomized rats that had measurable plasma prolactin or corticosterone levels, respectively, were excluded from the experiments. Frozen brains and pituitary glands were stored at −70°C and were cut in a cryostat within 2 days into 16-μm-thick tissue sections containing the following regions: posterior portion of the paraventricular nucleus, which contains the medial parvocellular and the posterior magnocellular subnuclei [bregma − 1.8 mm, according to Paxinos and Watson (26)]; central portion of the subfornical organ (bregma − 0.92 mm); and the pituitary gland through both the anterior and posterior pituitary.

Angiotensin-receptor binding was determined according to the method described by Israel *et al.* (5), by use of a single 2.5 nM concentration of ^{125}I-labeled [Sar^1]angiotensin II (Peninsula Laboratories, iodinated by DuPont/New England Nuclear; specific activity 2200 Ci/mmol; 1 Ci = 37 GBq). Nonspecific binding was determined by incubating adjacent tissue sections in the presence of 5 μM unlabeled angiotensin II. To determine whether the differences in ^{125}I-labeled [Sar^1]angiotensin II binding were due to alterations in the binding affinity or in the receptor number, a saturation experiment was performed in the parvocellular subnucleus of the paraventricular nucleus of six hypophysectomized and sham-operated rats. Serial sections cut through the paraventricular nucleus were incubated as described above with seven radiolabeled [Sar^1]angiotensin II concentrations ranging from 0.1 to 3.8 nM.

After incubation and washing, dry labeled sections were apposed against LKB ^{3}H-sensitive Ultrofilm (LKB) for 2–4 days depending on the receptor density. Sections from the same brain regions of all experimental groups were exposed on the same sheet of film together with a set of 16-μm-thick brain paste standards containing known quantities of ^{125}I-labeled ligand (5). The optical densities of the autoradiograms and standards were determined by RAS R1000 computerized densitometry apparatus (Amersham) and converted to fmol of ^{125}I-labeled angiotensin II bound/mg of standard protein. In the paraventricular nucleus, the magnocellular area was identified by comparison with an adjacent section stained with Luxol fast blue, and a mean value of nuclei in both sides was calculated. Nonspecific binding was subtracted from the total binding, and the results were expressed as mean ± SEM of specific binding. Statistical analyses were performed with one-way ANOVA, and P values of <0.05 were considered significant.

RESULTS

Angiotensin receptors were localized in the paraventricular nucleus, subfornical organ, and in the anterior pituitary gland, as described before (4, 5) (Figs. 1 and 2). Preliminary experiments could not detect significant angiotensin II binding in the supraoptic nucleus, and this nucleus was therefore not included in further angiotensin II-binding studies. In the paraventricular nucleus there was a high and uniform concentration of angiotensin receptors in the parvocellular area (Fig. 1). The magnocellular subnucleus showed only a low density of angiotensin receptors (Fig. 1). In the pituitary gland, angiotensin receptors displayed a patchy distribution throughout the anterior pituitary, but no angiotensin receptors were observed in the neurohypophysis (Fig. 2). Nonspecific binding was <10% of total binding in all areas investigated, except in the magnocellular paraventricular nucleus, where it was <25% of total binding.

Hypophysectomized rats had a lower density of angiotensin receptors in both magnocellular and parvocellular subnuclei of the paraventricular nucleus compared with sham-operated rats (Fig. 1, Table 1). Saturation experiments indicated the presence of only one class of binding sites in the parvocellular paraventricular nucleus and showed that the difference between the two groups was due to altered number of receptors, with no change in the binding affinity (Fig. 3). Hypophysectomy did not influence angiotensin receptors in the subfornical organ.

Adrenalectomy decreased angiotensin receptors in the subfornical organ as well as in both parvocellular and magnocellular paraventricular nucleus (Table 1). Corticosterone reversed the effects of adrenalectomy in all three nuclei, but mineralocorticoid replacement was effective only in the paraventricular nucleus. Angiotensin receptors in the pituitary gland were not altered by adrenalectomy.

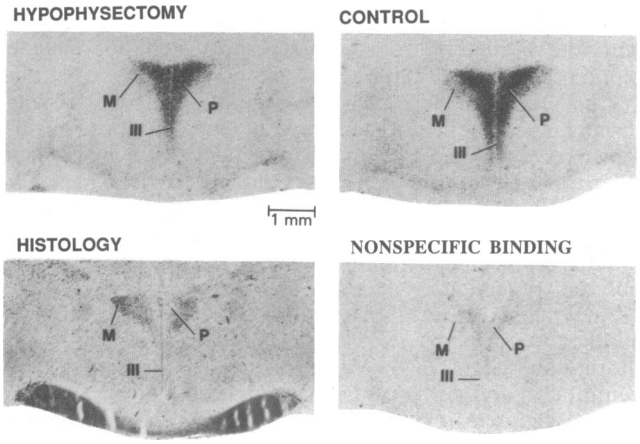

FIG. 1. Autoradiograms of angiotensin II receptors in the paraventricular nucleus of hypophysectomized and sham-operated rats. Tissue sections were incubated with 2.5 nM ^{125}I-labeled [Sar^1]angiotensin II. Section for histological localization was stained with Luxol fast blue. M, magnocellular; P, parvocellular subdivision; and III, third ventricle. (×6.)

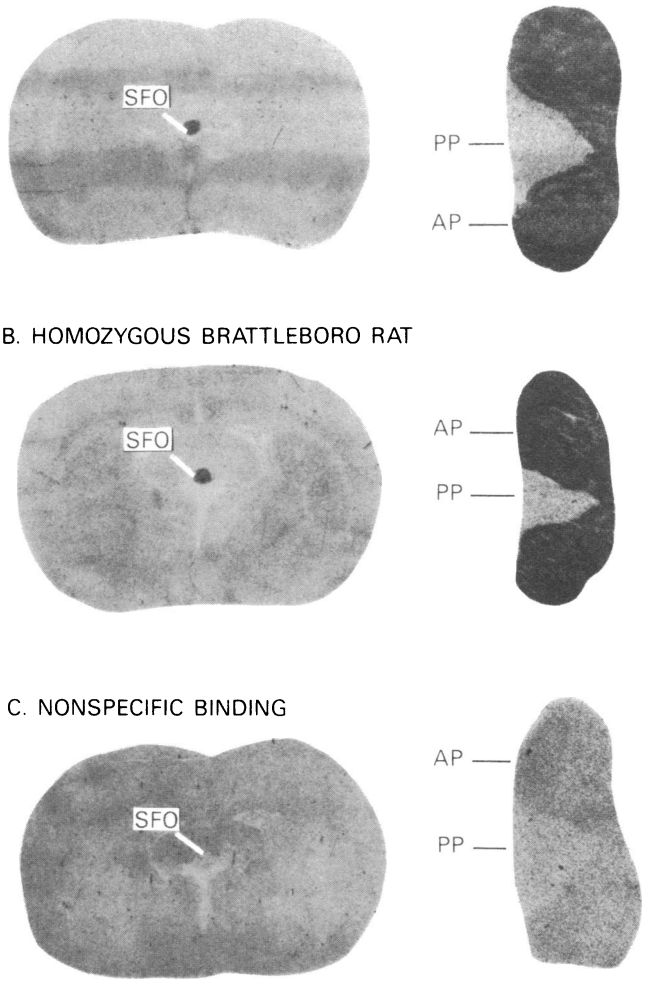

FIG. 2. Autoradiograms of angiotensin II receptors in the subfornical organ (SFO) (×3.5) and anterior (AP) and posterior (PP) pituitary gland (×7) of Long–Evans (*A*) and homozygous Brattleboro rats (*B*). Tissue sections were incubated with 2.5 nM ^{125}I-labeled [Sar^1]angiotensin II, and 1 μM of unlabeled angiotensin II was added to adjacent sections to determine the nonspecific binding (*C*).

Homozygous Brattleboro rats had a lower density of angiotensin receptors in the subfornical organ than Long–Evans rats (Fig. 2). Angiotensin receptor density was unaffected in both magnocellular and parvocellular paraventricular nucleus of Brattleboro rats. As described earlier (27), angiotensin-receptor density was higher in the anterior pituitary gland of both homozygous and heterozygous Brattleboro rats compared with the Long–Evans strain (Fig. 2, Table 1).

DISCUSSION

Our results indicate that angiotensin receptors in the paraventricular nucleus are under the control of corticosteroids. Hypophysectomized and adrenalectomized rats that have greatly reduced corticoid levels have a lower number of angiotensin receptors in the parvocellular portion of the paraventricular nucleus compared with sham-operated control rats. Both corticosterone and DOCA restored the receptor densities. Conversely, we have shown before that repeated stress, which is characterized by repeatedly elevated corticoid levels, increases angiotensin receptor densities in the parvocellular paraventricular subnucleus and in the subfornical organ (16). These data suggest that the activity of the subfornical organ–paraventricular nucleus connection may be increased in stress and decreased in the absence of corticoids. CRH release is, however, increased in both conditions (28, 29). The secretion of CRH is thus regulated by different factors in stress and after adrenalectomy. Angiotensin II might be one of the factors that maintain elevated CRH secretion during stress, when corticoids are high. When the corticoid production is abnormally low, other factors such as catecholamines and the lack of negative feedback by corticoids may control the stimulation of CRH secretion (30, 31).

Alterations in angiotensin receptors are not the only change observed in CRH neurons in the parvocellular paraventricular nucleus after adrenalectomy (for review, see ref. 13). CRH content is increased and vasopressin and angiotensin II, peptides that cannot be detected here in normal animals, are expressed in the same cells together with CRH (13). Glucocorticoid receptors have been shown to be localized within the same cells with CRH, and the regulation of the peptide content appears to be at least partially due to a direct effect of glucocorticoids on the gene expression in these cells (13).

The density of angiotensin receptors is decreased in the subfornical organ of adrenalectomized and vasopressin-deficient rats. Previous results from our laboratory show that angiotensin receptors in the subfornical organ are increased after chronic stress and dehydration (16, 17); plasma angiotensin II levels are high in all these conditions (19, 32, 33). The level of corticoids in the peripheral circulation is increased after dehydration and stress (19, 20) but absent or normal in adrenalectomized and Brattleboro rats, respectively (34). Plasma renin activity is within normal range after hypophysectomy (35), and angiotensin receptors in the subfornical organ are unaltered. Our data suggest that the response of angiotensin receptors in the subfornical organ to the increase

Table 1. Effects of endocrine manipulations on angiotensin II receptor concentrations

	n	SFO	PVN (parvocellular)	PVN (magnocellular)	Anterior pituitary
Control	12	308 ± 13	223 ± 10	56 ± 6	
Hypophysectomy	12	326 ± 17	175 ± 13*	30 ± 4*	
Control	12	365 ± 33	182 ± 7	43 ± 3	345 ± 30
Adrenalectomy	12	175 ± 22*	106 ± 8*	19 ± 1*	325 ± 35
ADX + corticosterone	8	379 ± 34	206 ± 12	46 ± 4	358 ± 59
ADX + DOCA	8	215 ± 30*	182 ± 19	40 ± 3	348 ± 60
Long–Evans	19	494 ± 25	190 ± 13	53 ± 6	346 ± 24
Brattleboro (homozygous)	19	327 ± 18*	212 ± 10	54 ± 9	457 ± 32*
Brattleboro (heterozygous)	8	451 ± 35	202 ± 7	55 ± 5	454 ± 53*

Tissue sections were incubated with 2.5 nM ^{125}I-labeled [Sar^1]angiotensin II. Results are reported as mean ± SEM fmol/mg of protein. SFO, subfornical organ; PVN, paraventricular nucleus; ADX, adrenalectomized.
*$P < 0.05$ against controls.

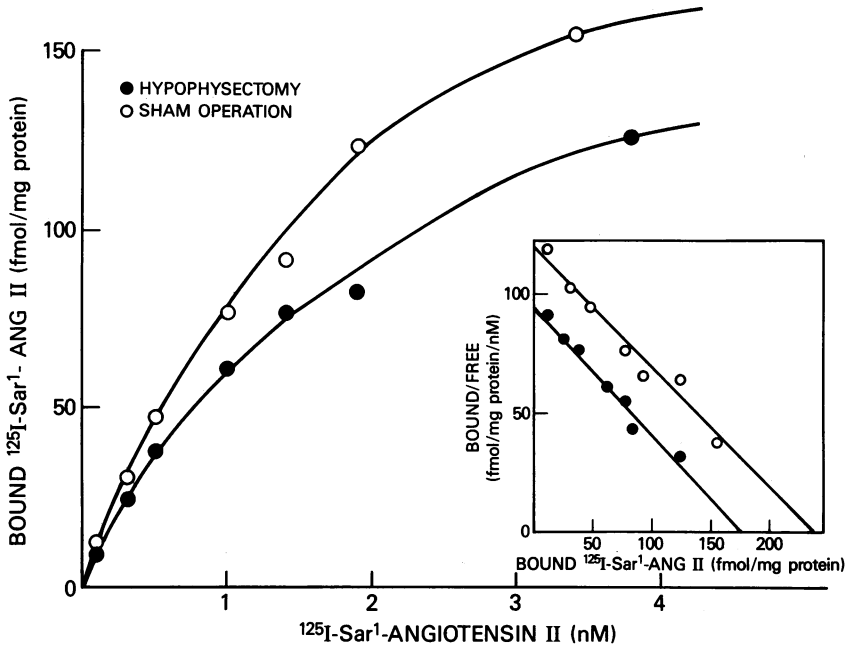

FIG. 3. Saturation curve and Scatchard plot (*Inset*) of ^{125}I-labeled [Sar^1]angiotensin II receptors in paraventricular nucleus of hypophysectomized (●) and sham-operated (○) rats. Each point represents the mean of nuclei from six individual rats. K_d, 1.85 and 1.97 nM; B_{max}, 176 and 241 fmol/mg of protein for hypophysectomized and sham-operated rats, respectively.

in plasma angiotensin II may depend on the plasma levels of corticoids.

Replacement with both corticosterone and DOCA reversed the effect of adrenalectomy on angiotensin-receptor binding in the paraventricular nucleus, but only corticosterone was effective in the subfornical organ. It is possible that corticosterone acts through mineralocorticoid receptors in the paraventricular nucleus, because corticosterone has significant mineralocorticoid activity; this is consistent with reports that mineralocorticoid excess increases brain angiotensin II receptor binding (21, 22).

Recent observations demonstrate that adrenalectomized rats are less sensitive to blood pressure-elevating effects of peripherally administered angiotensin II than sham-operated rats (32). The subsensitivity can be reversed by dexamethasone but not by aldosterone (32). This finding is consistent with our results showing that the lower number of angiotensin receptors in the subfornical organ of adrenalectomized rats could be reversed by glucocorticoid but not by mineralocorticoid replacement. Because the subfornical organ has been implicated in the central regulation of blood pressure (6), we suggest that the low number of angiotensin receptors in the subfornical organ and the subsensitivity to peripheral angiotensin II in adrenalectomized rats could be directly related. The regulatory effect of glucocorticoids on central angiotensin receptors might, therefore, be of physiological importance in the regulation of blood pressure.

Perikarya of the neurons that release vasopressin from the posterior pituitary gland lie in the supraoptic nucleus and magnocellular paraventricular subnucleus (13). Central administration of angiotensin II is known to stimulate vasopressin release from the posterior pituitary gland (2, 10), and electrophysiological studies suggest that angiotensin II might act directly on vasopressin neurons in these magnocellular nuclei (11). We have not, however, been able to detect significant quantities of angiotensin II-binding sites in the supraoptic nucleus, and binding-site levels in the magnocellular paraventricular nucleus are much lower than those in the parvocellular subnucleus. Moreover, alterations in the angiotensin-receptor density in the magnocellular paraventricular nucleus were always coupled with concomitant change to the same direction in receptor density in the parvocellular area. Because some parvocellular cells are localized in the magnocellular subnucleus (13), it is possible that angiotensin receptors detected in the magnocellular paraventricular nucleus might be located on parvocellular cells. Finally, magnocellular angiotensin II-binding sites were not altered by the lack of vasopressin in Brattleboro rats. Although the evidence is indirect, these results do not lend support to the hypothesis that angiotensin II increases vasopressin secretion by acting directly through angiotensin receptors on vasopressin cells.

Angiotensin receptors in the anterior pituitary gland seem to be sensitive to dehydration, because they are increased in chronically dehydrated Brattleboro rats but also in acutely dehydrated normal rats (27, 36). It has been suggested that angiotensin II might contribute to the stimulation of ACTH secretion in the pituitary gland, either by itself of by potentiating the effects of CRH (8, 37). We did not observe alterations in the density of angiotensin receptors in the pituitary gland after adrenalectomy, although this condition decreases CRH receptors >70% (28). Moreover, these receptors were not altered by stress, whereas brain receptors were increased (16). These results demonstrate that pituitary angiotensin receptors, contrary to those in the brain, do not seem to be regulated by corticoids and indirectly support the hypothesis that the site where angiotensin II contributes to the stimulation of ACTH release *in vivo* is not at the pituitary corticotrophs, but more probably at CRH neurons in the paraventricular nucleus.

It is of interest to compare the regulation of angiotensin and CRH receptors in brain and pituitary gland after adrenalectomy and chronic stress. Both stress and adrenalectomy diminish CRH receptors in the anterior pituitary gland but not in the brain (28, 29). Because CRH secretion is increased in both these conditions, CRH may be the most important factor to regulate CRH receptors in the pituitary gland (29). Angiotensin receptors, on the other hand, are increased after stress (16) and decreased after adrenalectomy in the subfornical organ and the paraventricular nucleus but remain unaltered in the pituitary gland. The difference in the influence of stress and adrenalectomy on CRH and angiotensin receptors indi-

cates different physiological roles for these hormones in the regulation of ACTH secretion. Whereas CRH appears to directly increase ACTH secretion in pituitary corticotrophs, brain angiotensin II may have a corticoid-dependent modulatory role on CRH secretion, which could be of physiological importance in the adaptation to states with altered CRH secretion, such as chronic stress.

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