Pregnancy-induced changes in central response to atrial distension mimicked by progesterone metabolite

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Deng, Yiming, and Susan Kaufman. Pregnancy-induced changes in central response to atrial distension mimicked by progesterone metabolite. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1875–R1877, 1998.—In virgin female rats, atrial distension (an index of blood volume expansion) causes an increase in c-fos expression in the paraventricular nucleus of the lateral hypothalamus. During pregnancy, this response is markedly attenuated. We tested the effects of 3α-hydroxy-5α-pregn-20-one (3α-OH-DHP) on activation of central pathways following stimulation of the atrial volume receptors. Not only does this progesterone metabolite increase during pregnancy, but it has already been implicated in pregnancy-induced changes in the baroreflex. Female rats were prepared with indwelling venous cannulas and intracardiac balloons that, when inflated, caused a discrete localized stimulation of the atrial volume receptors in the absence of changes in cardiac hemodynamics. Seven days later, the rats were infused with 3α-OH-DHP dissolved in cyclodextrin and the intracardiac balloons were inflated. One hour later, the rats were killed and fixed by perfusion and the brains were prepared for visualization of c-fos activity. Infusion with 3α-OH-DHP significantly reduced the central response to atrial distension, i.e., it mimicked pregnancy. These results are consistent with the suggestion that this metabolite of progesterone may be an important factor in cardiovascular adaptation to pregnancy.

allopregnalone; blood volume; baroreceptor; baroreflex

CIRCULATING BLOOD VOLUME is monitored by mechanoreceptors situated at the junctions of the great veins and the heart (16). In response to volume expansion, neural and hormonal responses that increase urine output are elicited. If the atrial volume receptors are selectively stimulated in male or nonpregnant female rats, there is an increase in urine volume and sodium and potassium output (12). During pregnancy, this response is abolished (13). Moreover, it has been shown that during pregnancy, there is an attenuated depressor response to volume-induced increases in right atrial pressure (8). We propose that it is this inability of the volume receptors to initiate a reflex homeostatic response that allows blood volume to increase so markedly (40%) during pregnancy (9, 17).

We have shown that failure to respond to atrial distension during pregnancy lies with both the hormonal and the neural arms of the reflex (5, 13, 24). In vivo and in vitro experiments have revealed that secretion of atrial natriuretic peptide is inhibited (13, 24). Moreover, activation of neurons in the lateral hypothalamus, which is normally observed after atrial distension, does not occur in pregnant animals (5). We sought to determine what factor(s) might be responsible for these pregnancy-induced changes in the neural response to volume expansion. Specifically, we investigated the effect of the progesterone metabolite 3α-hydroxy-5α-pregn-20-one (3α-OH-DHP) on activation of neurons in the lateral hypothalamus following localized distension of the atrial volume receptors.

MATERIALS AND METHODS

Animals. Female Long-Evans rats (250–300 g) were obtained from Charles River Canada (St. Foy, PQ, Canada). Before surgery, they were held for at least 1 wk in a temperature- and humidity-controlled room with a 12:12-h light-dark cycle (light 0700–1900). After surgery, the rats were allowed to recover their preoperative weights before the experiments began. They were maintained on a 0.28% sodium diet (PMI Feeds, St. Louis, MO).

Surgery. All the rats were prepared (under pentobarbital sodium anesthesia, 62 mg/kg body wt ip) with a small inflatable balloon located at the right venoatrial junction. Briefly, the balloon was passed down the right jugular vein and positioned at the right superior vena caval-right atrial junction. The methods have previously been described in detail (11). Inflation of the balloon with 50 µl saline yielded a diameter of ~5 mm. Visually, we have confirmed that this causes the venoatrial junction to be gently distended. The peculiar anatomy of the heart, whereby blood from the left jugular vein enters the inferior vena cava, enables one to stretch the venoatrial junction without interfering with venous return to the heart. (Blood drains from the head into the left superior vena cava via cross circulation in the head and neck.) There are no accompanying changes in central venous pressure, atrial pressure, or mean arterial pressure when the balloon is inflated (11, 14). Indwelling cannulas were also placed nonocclusively in the inferior vena cavae (10). The cannulas, which were connected to stainless steel tubing secured to the interscapular region, were used to administer the steroid.

Experimental protocol. Seven days after surgery, the rats were randomly allocated to the following groups: 1) rats infused intravenously with 3α-OH-DHP and subjected to atrial distension (n = 6), 2) rats infused with vehicle and subjected to atrial distension (n = 7), 3) rats infused with vehicle with no atrial distension (n = 4), and 4) rats infused with 3α-OH-DHP with no atrial distension (n = 6). All the rats were placed in metabolism cages the day before testing for ease of accessing the cannulas. The next day, groups 1 and 4 received a bolus of 3α-OH-DHP (50 µg in 0.1 ml solvent), followed by an infusion at a rate 0.5 µg·10 µl−1·min−1. The intracardiac balloons in groups 1 and 2 were inflated with 50 µl saline; the balloons in the control groups 3 and 4 were not inflated.

One hour later, the rats were anesthetized (pentobarbital sodium, 62 mg/kg iv). A few minutes later, perfusion was started through the left ventricle, first with heparinized 0.9% saline (ice cold, 100 ml in 5 min), followed by a solution of 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2 (ice cold, 400 ml in 35 min). The whole brains were removed and postfixed for 1 h in 4% paraformaldehyde at 4°C and then 20%
sucrose (in water) overnight at 4°C. The following day, the brains were transferred into 30% sucrose (in water) for 1 h. Coronal sections (50 µm) through the whole brain were cut in a cryostat, and every other section was collected in PBS (pH 7.2).

Immunocytochemistry. Sections were incubated overnight in cold anti-Fos antiserum (2 µg/ml in 0.3% Triton X-100 in PBS, Ab-2, polyclonal rabbit IgG, cat no. PC05, Oncogene Science). According to the supplier's specifications, this polyclonal antibody reacts with both cellular and viral forms of Fos, but does not react with the 39,000 molecular weight Jun protein (first antibody was purified on peptide columns). Tissues were sequentially incubated for 1 h with anti-rabbit IgG (1:200 in PBS, biotinylated antibody, Vectastain, Vector Laboratories), followed by ABC reagent for another hour, (1:100 in PBS). To visualize Fos, sections were treated with 0.05% diaminobenzidine (Sigma) solution containing 0.01% hydrogen peroxide in PBS for 5–10 min until they turned brown. Control sections taken from the selected nuclei were processed in exactly the same manner, except the primary antibody was omitted. No immunoreactivity was observed.

Preparation of drugs. 3α-OH-DHP (Sigma Chemical, Mississauga, ON, Canada) was dissolved in 20% 2-hydroxypropyl-β-cyclodextrin (Sigma Chemical) in sterile water.

Quantitative analysis. Cell nuclei in the medial preoptic area were examined, and the number of Fos-labeled cells was counted in every other section throughout the whole nucleus (4–5 sections). The total number of neurons expressing c-fos was recorded. Values were expressed as mean number ± SE per nucleus. Statistical multiple comparisons between the groups were evaluated using ANOVA. Student-Newman-Keuls method was then used to determine which group(s) contributed to these differences. Significance was accepted at \( P < 0.05 \).

**RESULTS**

Neither vehicle nor 3α-OH-DHP altered basal expression of Fos in the paraventricular nucleus. Distension of the right superior vena caval-right atrial junction of rats infused only with vehicle caused a significant increase in Fos-positive neurons in the paraventricular nucleus (Figs. 1 and 2). This response was significantly attenuated in the steroid-treated animals.

**DISCUSSION**

Expression of the immediate-early gene c-fos has been used and validated as a marker for neural systems activated by a variety of stimuli (4, 15, 20). Key sites for integration of cardiovascular homeostasis through the endocrine and neural systems are located in the hypothalamus. This area possesses reciprocal connections with the midbrain as well as efferent projections to the neurohypophysis. Specifically, this region receives projections from the nucleus of the solitary tract, that region of the brain believed to receive input from the volume and pressor receptors (4). We have previously shown that, whereas atrial distension normally results in activation of hypothalamic neurons, this response is absent in pregnant animals (5). The data presented in this paper show the response to be similarly attenuated by the progesterone metabolite 3α-OH-DHP.

During pregnancy, both brain and plasma levels of 3α-OH-DHP increase (19). Interest in this steroid has, in the past, been directed primarily toward its sedative-hypnotic activity in the central nervous system (2, 23). It is believed to act by binding to receptors for the inhibitory neurotransmitter GABA (19). Because GABAergic neurons are implicated in sympathoinhibitory pathways in the brain, there has also been some interest in the role of these neuroactive steroids in control of the cardiovascular system (6, 22).

Masilamani and Heesch (18) have recently demonstrated that 3α-OH-DHP mimics pregnancy with respect to the reflex response to changes in blood pressure; although baseline MAP was unchanged, the baroreflex sympathoexcitatory responses were de-
increased (18). Our results suggest that control of blood volume may be similarly affected. Like pregnancy, 3α-OH-DHP attenuates the central response to atrial distension. Thus, as blood volume increases during pregnancy, those mechanisms that would normally be activated to limit that increase are disabled. This increase in blood volume appears to be critical to a successful pregnancy. There is a strong inverse correlation between blood volume and fetal growth retardation, and one of the most significant diseases of pregnancy, pregnancy-induced hypertension/preeclampsia, is characterized by a reduction in blood volume (7, 21).

Perspectives

Our results suggest that the complex integration of hormonal changes (antidiuretic hormone release), behavioral changes (drinking), and sympathetic outflow (renal output and renin release) that would normally occur in the lateral hypothalamus as a result of atrial distension is altered during pregnancy. It is not known whether this reflects a failure of the volume receptors to detect the change in atrial size (a change in the transducer properties of the receptor) or whether the signals coming from the receptors are differently processed in the central nervous system during pregnancy. Given that brain levels of 3α-OH-DHP are known to increase during pregnancy (19), and given that the neuroactive steroids are known to interact with GABA (19), it is tempting to speculate that inhibitory GABA-mediated mechanisms might be involved in the attenuation of both the pressor and volume reflex pathways. Indeed, there is evidence that tonic stimulation of GABA receptors in the nucleus of the solitary tract attenuates the baroreceptor reflex (6, 22). Thus inhibitory pathways from the nucleus of the solitary tract to the lateral hypothalamus, which would normally be subject to regulatory control by input from the pressor and volume receptors, might be tonically activated during pregnancy by stimulation of the GABA receptors by high circulating levels of 3α-OH-DHP. Such a mechanism could then account for the increased salt and water intake (1), increased blood volume (9, 17), and attenuated arterial baroreflex (3, 18) characteristic of pregnancy.

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