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17. Monocyte cultures were examined for cell morphology, ability to ingest carbon particles, and uptake of nonspecific esterase stain. More than 99 percent of the cultured monocytes stained positive with monoclonal antihuman monocyte antibody 61D3 (Bethesda Research Laboratories). Culture medium consisted of RPMI 1640 supplemented with 5 percent heat-inactivated human type AB serum. Human lymphokine preparations containing MAF were produced by incubating human mononuclear leukocytes with Sepharose-bound concanavalin A (Pharmacia) for 48 hours and separating the supernatants by centrifugation.
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20. We thank S. Buckner for secretarial assistance. Human recombinant γ-INF was supplied by Genentech (South San Francisco, Calif.), and MTP-PE was supplied by Ciba-Geigy (Basel, Switzerland). Sponsored by the National Cancer Institute, Department of Health and Human Services, under contract No. NO1-CO-23909 with Litton Bionetics.
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Androgen Modulation of Adrenal Angiotensin Receptors

Abstract. *Several polar androgens increased the binding of angiotensin and its stimulation of aldosteronogenesis in bovine adrenal glomerulosa cells. The effect was seen only if the steroids were applied to the cells and then washed away. This phenomenon and the technique for demonstrating it may have implications for studies of receptor modulation and for clinical states in which responsiveness to angiotensin is increased.*

In several forms of hypertension, including the majority of patients with low renin essential hypertension (LREH), adrenal secretion of aldosterone in response to angiotensin II (AII) is exaggerated (*1–3*). One possible mechanism for this hyperresponsiveness is an increase in the number or affinity of angiotensin receptors (*4*). We have observed receptor modulation in bovine adrenal glomerulosa cells exposed to cholesteryl hemisuccinate (*5*). Because androgens have been implicated in the pathogenesis of some forms of hypertension (*6–8*), we examined the effects of several androgens on angiotensin receptors and responses in bovine cells.

Collagenase-dispersed adrenal glomerulosa cells from mature cows (*9*) were suspended in Krebs solution containing 20 m*M* Hepes (Sigma). To cell suspensions were added testosterone hemisuccinate (Steraloids), dehydroepiandrosterone (DHEA) sulfate (Sigma), or their ethanol vehicle (1 percent by volume). Androgen concentrations ranged from 8.6×10^{-8} to $8.6 \times 10^{-4}M$. Cells were incubated with these agents for 2 to 60 minutes at room temperature in a shaking water bath and then washed twice with buffer (*5*). Equal numbers of cells were used in all groups, and there was no between-group difference in cell viability as determined by trypan blue exclusion.

Angiotensin II and angiotensin III (AIII) (Bachem) were labeled with ^{125}I (*10*). Binding to cell suspensions was measured by using the method of Carroll *et al.* (*5*), except that EDTA (15 m*M*) and dithiothreitol (1 m*M*) were added to retard peptide degradation. Binding of AII and AIII was also measured in the presence of testosterone hemisuccinate added in ethanol (1 percent by volume) to a final concentration of $8.6 \times 10^{-4}M$. Binding experiments were performed at 37°C for 15 (AIII) or 30 (AII) minutes. Saturable binding was defined as the difference between total binding and binding in the presence of 10 μg of unlabeled angiotensin. Binding results were expressed as percentages of control values in each experiment and were subjected to Scatchard analysis.

Effects of testosterone hemisuccinate on aldosterone secretion by bovine cells were determined in quintuplicate (*5*) by direct radioimmunoassay for aldosterone with [^{125}I]aldosterone and antibody to aldosterone (Diagnostic Products). Extraction and chromatography of selected samples with Sephadex LH-20 (Pharmacia Fine Chemicals) did not affect the results. We used angiotensin concentrations of 10^{-9} and $10^{-7}M$ in steroidogenesis experiments because at those levels AII produced submaximal and maximal aldosterone responses, respectively (*5, 9, 11, 12*). Statistical analysis was performed with Student's *t*-test for unpaired values.

When testosterone hemisuccinate was present in the medium during binding

Table 1. Equilibrium binding parameters for angiotensins and bovine adrenal glomerulosa cells. Values are derived from analysis of the data in Figs. 1 and 2 with Scatfit, the nonlinear least-squares program developed by Rodbard (*28*), and are presented as means ± standard errors.

Parameter*	Control		Testosterone hemisuccinate	
	Affinity (nM^{-1})	Capacity (femtomoles per 10^6 cells)	Affinity (nM^{-1})	Capacity (femtomoles per 10^6 cells)
	Angiotensin II			
K_1	1.87 ± 0.61		3.04 ± 0.47	
Q_1		81.2 ± 38.1		104 ± 25
K_2	0.101 ± 0.058		0.278 ± 0.096	
Q_2		653 ± 173		351 ± 38
	Angiotensin III			
K_1	10.7 ± 3.6		4.42 ± 1.20	
Q_1		15.4 ± 3.8		82.2 ± 24.6
K_2	0.0907 ± 0.0235		0.162 ± 0.088	
Q_2		603 ± 115		522 ± 150

*K_1 and K_2 are the affinity constants and Q_1 and Q_2 are the corresponding capacities for each of two independent classes of binding sites.

determinations with bovine glomerulosa cells (three experiments), binding of AII and AIII was inhibited 51.6 percent ($P < 0.001$) and 7.3 percent (not significant), respectively. By contrast, when cells were incubated with the steroid derivative and then washed, testosterone hemisuccinate caused a striking increase in AII binding. Treated cells bound 161.3 ± 9.8 percent of the amount of [^{125}I]AII and 222 ± 14 percent of the amount of [^{125}I]AIII bound by control cells (means ± standard errors for 11 experiments; $P < 0.001$). Enhanced angiotensin binding was observed after as few as 2 minutes of incubation with testosterone hemisuccinate; longer incubation times were not associated with further changes in binding. Comparable increases in binding were also seen with tritiated angiotensins. The ethanol vehicle increased angiotensin binding less than 10 percent. High-performance liquid chromatography showed that intact [^{125}I]AII accounted for two-thirds of the bound label in both control and testosterone hemisuccinate–treated cells incubated with AII.

Three experiments measuring AII binding were analyzed by the Scatchard method; Fig. 1A and Table 1 depict the results of one of those experiments. Testosterone hemisuccinate enhanced binding by increasing receptor number and affinity. The effects on receptor number were more consistent. This was most evident in an experiment with AIII (Fig. 1B and Table 1).

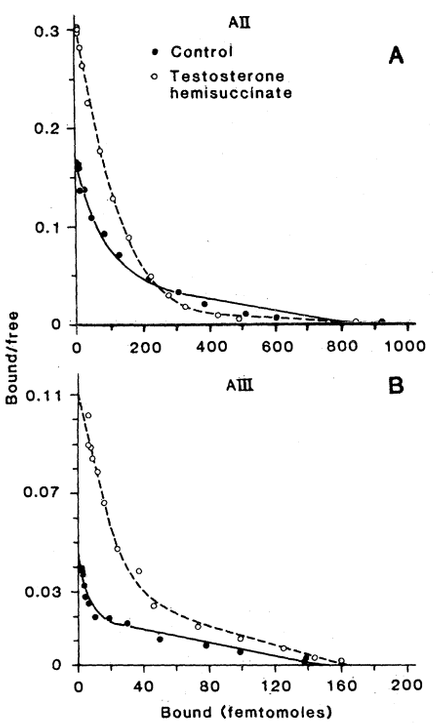

Fig. 1. Effect of testosterone hemisuccinate ($8.6 \times 10^{-4}M$) on angiotensin binding in bovine adrenal glomerulosa cells. (A) Scatchard plot for AII binding. (B) Scatchard plot for AIII binding.

Steroidogenic responses to 10^{-9} and $10^{-7}M$ AII were significantly increased in bovine cells after incubation with testosterone hemisuccinate (Fig. 2A). The enhanced response was more noticeable with the $10^{-9}M$ concentration of AII. Testosterone hemisuccinate also significantly raised basal aldosteronogenesis. A similar pattern of response was seen in experiments with AIII (Fig. 2B).

In the experiments described above testosterone hemisuccinate was used at a final concentration of $8.6 \times 10^{-4}M$, five orders of magnitude greater than the physiological concentration of testosterone in human peripheral blood. Treatment of cells with testosterone hemisuccinate at $8.6 \times 10^{-8}M$ had smaller, nonsignificant effects on angiotensin binding but significantly affected angiotensin-stimulated secretion of aldosterone. In three experiments with AII, binding increased 6.8 percent after steroid exposure compared to the control value. However, aldosterone secretion increased 56.7 and 38.1 percent with 10^{-9} and $10^{-7}M$ AII, respectively ($P < 0.001$). AIII binding rose 12.6 percent in cells treated with testosterone hemisuccinate at $8.6 \times 10^{-8}M$ in three experiments; aldosteronogenesis increased 20.8 percent ($P < 0.001$) and 11.8 percent ($0.1 > P > 0.05$) in response to AIII at 10^{-9} and $10^{-7}M$, respectively.

Since testosterone hemisuccinate is not a naturally occurring steroid, we examined the effects of DHEA sulfate. In two experiments, bovine adrenal cells were incubated for 60 minutes with this endogenous androgen at $8.6 \times 10^{-5}M$, a concentration one order of magnitude greater than that found in peripheral human blood (300 μg per 100 ml). Cells were then washed and angiotensin binding and steroidogenesis were measured. DHEA sulfate increased AII binding 16.7 percent over the control value ($P < 0.05$). In response to 10^{-9} and $10^{-7}M$ AII, aldosterone secretion increased 31.8 percent (not significant) and 27.2 percent ($P < 0.05$), respectively. Basal aldosteronogenesis was increased 19.8 percent ($0.1 > P > 0.05$).

Among other sterols and steroids tested, cholesteryl hemisuccinate markedly inhibited angiotensin binding (*5*) while androsterone hemisuccinate, hydrocortisone hemisuccinate, testosterone, and androstenedione had no effect when binding was measured after these compounds were removed from the medium.

Bovine adrenal glomerulosa cells washed after incubation with testosterone hemisuccinate displayed marked increases in AII and AIII binding that were not seen when the steroid was present during the binding assay. The increase in binding was associated with significantly enhanced responses of aldosteronogenesis to AII and AIII. The rise in basal aldosteronogenesis in cells exposed to testosterone hemisuccinate suggests a response to angiotensins produced by the cells themselves. This hypothesis must be considered in view of the presence of renin in adrenal tissues (*13*).

Sterols and steroids may alter cell membrane characteristics such as fluidity (*14, 15*). We observed decreased fluidity of bovine adrenal glomerulosa cell membranes after incubation with cholesteryl hemisuccinate (*5*). The change in fluidity was accompanied by an 84 percent inhibition of AII binding, but effects of AII on aldosterone secretion were preserved. In other ligand-receptor systems, changes in fluidity caused either parallel (*16–21*) or inverse (*22–24*) shifts in receptor number. Testosterone hemisuccinate could have acted on membrane properties other than fluidity. In mouse

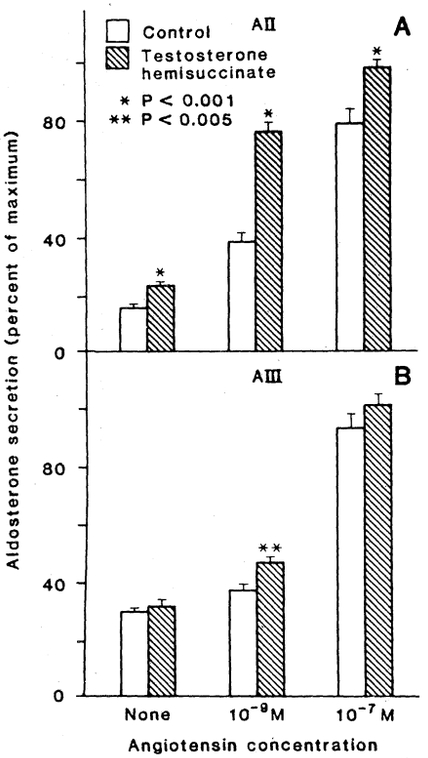

Fig. 2. Effect of testosterone hemisuccinate ($8.6 \times 10^{-4}M$) on stimulation of aldosterone secretion in bovine adrenal glomerulosa cells. (A) Basal and AII-stimulated aldosterone production. Values are means ± standard errors for seven experiments ($n = 28$ or more for each bar). (B) Basal and AIII-stimulated aldosteronogenesis. Values are means ± standard errors for four experiments ($n = 19$ or more for each bar).

LM cells, manipulating the membrane phospholipid composition modulated adenylate cyclase activity independently of changes in fluorescence polarization, a measure of membrane fluidity (*25*). A change in receptor synthesis or degradation by testosterone hemisuccinate is unlikely to account for its effects, because these effects occurred rapidly and included a shift in receptor affinity.

Our observations indicate possible pitfalls in investigating the effects of proposed receptor modulators on the actions of angiotensin or other ligands. For example, approximately half of the 70 steroids we added to adrenal cells inhibited angiotensin binding (*26*). However, in all of those experiments the steroids remained in the medium during the binding assay. Tested in the same way, testosterone hemisuccinate also inhibited angiotensin binding. However, in cells washed thoroughly after incubation with this steroid, angiotensin binding and stimulation of aldosteronogenesis were markedly augmented. Putative modulators may exert complex positive and negative effects or may require a latent period to influence receptors. Investigators testing substances for modification of membrane-related processes should take these possibilities into account.

Our ability to enhance the activity of angiotensin receptors by naturally occurring steroids in vitro offers a new way to test for putative mediators of low renin essential hypertension. This condition is usually marked by normal aldosterone secretion in the face of subnormal levels of renin (*27*) and may be the result of sensitization of adrenal glomerulosa by circulating substances. We suggest that angiotensin potentiators in body fluids from patients with low renin essential hypertension can be sought by a brief, transient exposure of adrenal cells to the fluids, as well as by adding the fluids to the final assay mixture.

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Perinatal Dopamine–Related Drugs Demasculinize Rats

Abstract. *Administration of haloperidol, a common neuroleptic, to pregnant or lactating rats impaired the masculine sex behavior of their male offspring. Prenatal haloperidol did not affect testosterone concentrations in fetuses. Maternal administration of apomorphine, a dopamine agonist, and of α-methyl-*p*-tyrosine, an inhibitor of dopamine synthesis, also demasculinized male offspring. In both experiments other behaviors and developmental milestones were unaffected. Perinatal haloperidol, apomorphine, and α-methyl-*p*-tyrosine did not lower testosterone in adulthood. These drugs may act directly on neurons that control masculine behavior without lowering testosterone prenatally or in adulthood.*

Perinatal administration of certain drugs or hormones produces lasting impairment of reproductive function and sexual behavior in male rodents (*1–4*). Several of these agents appear to exert their demasculinizing effects by reducing the concentration of testosterone perinatally or in adulthood (*2, 3, 5, 6*). While high concentrations of testosterone during the week before and the week after birth appear to be critical for sexual differentiation (*7*), the mechanisms by which testosterone exerts its masculinizing effects have not been identified.

One possible mechanism of testosterone action involves a direct effect on developing neurons through alterations in growth, synaptogenesis, receptors, or enzyme activity. Monoaminergic neurons have been shown to regulate the expression of sex behavior in adulthood, with dopamine (DA) facilitating (*8, 9*) and serotonin inhibiting masculine behavior (*9, 10*). Alterations in monoaminergic activity may also play a role in the developmental demasculinizing effects noted above. Because DA is the only monoamine shown to facilitate masculine behavior in adulthood, we investigated the effects of several perinatally administered drugs that affect DA transmission.

Haloperidol (HAL) blocks DA receptors preferentially though not exclusively and crosses placental and lactational barriers (*11*). Rosengarten and Friedhoff (*12*) reported that administration of HAL to pregnant rats depressed binding of [^{3}H]spiroperidol in the brains of their offspring as late as 60 days of age. Behavioral responsiveness of the offspring to apomorphine (APO), a DA agonist, was also depressed by prenatally administered HAL. On the other hand, neonatal treatment with HAL (through lactation) had the opposite effect, increasing [^{3}H]spiroperidol binding and behavioral responsiveness to APO. This is similar to the supersensitivity to DA seen in adult animals after long-term treatment with neuroleptics. Because DA facilitates adult masculine sex behavior, we hypothesized that prenatally administered HAL would impair masculine behavior in adulthood, that neonatal treatment with HAL would facilitate it, and that combined pre- and neonatal treatments would tend to cancel these effects.

Ten Long-Evans female rats were mated and divided into two groups that were injected intraperitoneally with HAL (2.5 mg/kg) or saline from day 7 of gestation until day 21 postpartum, except on the day of birth. Treatment parameters were the same as those shown by Rosengarten and Friedhoff (*12*) to