compounds and may facilitate the screening of agents active against other viruses and the more detailed study of the therapeutically active anti-viral agents now known.

1. Kaufman, H. E., Nesburn, A. B., Maloney, E. D., *Arch. Ophth.*, 1962, v67, 583.

2. Kaufman, H. E., Martola, E.-L., Dohlman, C.-H., *ibid.*, 1962 v68, 235.

3. Enders, J. P., Peebles, T. C., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 277.

4. Stocker, M. G. P., *Virus Growth and Variation,* University Press, Cambridge, 1959.

5. Kaufman, H. E., Maloney, E. D., Nesburn, A. B., *Investigative Ophthal.*, 1962, v1, 686.

6. Tamm, I., *Clin. Pharmacol. & Therap.*, 1960, vL, 779.

7. Herrmann, E. C., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 142.

Received September 13, 1962. P.S.E.B.M., 1963, v112.

Further Evidence for a Role of the Renin-Angiotensin System in Regulation of Aldosterone Secretion.* (27933)

PATRICK J. MULROW, WILLIAM F. GANONG, AND ANGELA BORYCZKA
Department of Medicine, Yale University School of Medicine, New Haven, Conn. and Department of Physiology, University of California School of Medicine, San Francisco

Previous studies(1-7) have shown that saline extracts of dog kidneys, relatively crude preparations of renin, and synthetic valine-5-angiotensin II increase aldosterone secretion in nephrectomized hypophysectomized dogs. Another pressor substance, norepinephrine, had no effect upon aldosterone secretion. Further evidence for the specificity of the renin-angiotensin system in stimulating aldosterone secretion has now been obtained by measuring the effect of purified hog renin preparations, human renin, erythropoietin and a non-pressor synthetic analogue of angiotensin II on aldosterone secretion. The effect of a carboline (1-methyl-6-methoxy 1, 2, 3, 4, tetrahydro-2-carboline), presumably manufactured in the pineal gland(8), has also been measured in nephrectomized hypophysectomized dogs. This carboline has been claimed by Farrell and McIsaac(8) to be a potent stimulator of aldosterone secretion.

Methods. Mongrel dogs were hypophysectomized and/or nephrectomized, the right lumbar adrenal vein cannulated and aldosterone and 17-hydroxycorticoids measured in adrenal venous effluent by technics described previously(3). Hog renin preparations of 2 different purities were injected over one minute. One preparation contained 24 units per mg of protein and the other 3 units per mg. Adrenal venous blood was collected immediately before and from 4 to 14 minutes after start of the injection. Two human kidneys were extracted separately for renin by the method previously described(3) and each was injected as a single injection. Two erythropoietin preparations were injected in the same way as the renin preparation. One hundred and fifteen comparative standard units (9) erythropoietin prepared from human urine (activity 2.3 CS units per mg) and 7.2 comparative standard units erythropoietin prepared from plasma of phenylhydrazine treated sheep (Armour A2-0336) were injected. Angiotensin II diamide (aspartyl-1, valine-5, phenylalanyl-8 diamide) in small volumes of saline was infused for 14 minutes as described previously for angiotensin II(3). Adrenal venous blood was collected immediately before start of the infusion and from 4 to 14 minutes during the infusion. 1 Methyl-6-methoxy 1, 2, 3, 4, tetrahydro-2-carboline was infused for 2 hours in 10% ethanol in saline or ascorbic acid in saline *via* a constant infusion pump. A concentrated solution was made up fresh from crystalline material in the dark at 4°C and at half-hour intervals dilutions were made for infusion. All syringes were covered with aluminum foil to prevent possible destruction by light. From 4 to 10 μg per hour was infused over 2 hours in a total infusate volume of 200-225 ml. Following 2 control adrenal venous samples, 3 to 4 samples of adrenal venous blood were

* This work was supported by grants from U. S. Public Health Service and Commonwealth Fund.

TABLE I. Renin and Erythropoietin Experiments.

Preparation	Dose	△ BP mm Hg	Aldosterone secretion (mμg/min) Control	Post Injection
Hog renin	.7 mg*	75/70	1	20
" "	.7 " *	50/40	0	12
" "	.04 " †	35/30	0	32
" "	.04 " †		0	7
Human renin	5.3 g‡	20/20	0	11
" "	13.3 "‡	70/60	1	42
Dog renin (mean of 19 injections)	.6 to 4.9 mg§	23/22	5	20 ($P < .001$)
Erythropoietin (sheep plasma 7.2 CS units)		0	7	5
" (human urine 115 CS units)		0	9	5

* Mg of protein. Renin conc. 3 units/mg protein.
† " " " " " 24 " " " "
‡ Wet weight of kidney from which renin was extracted.
§ Mg of protein.

collected at half-hour intervals during infusions. Carboline was infused into 5 hypophysectomized dogs and 3 hypophysectomized-nephrectomized dogs. In all experiments hypophysectomy was judged to be complete by the low control levels of 17-hydroxycorticoids and by the absence of pituitary remnants upon gross inspection of the sella turcica and hypothalamus at completion of the experiment. Femoral arterial pressure was monitored continuously during all experiments by means of a Statham strain gauge and a Grass model 5 polygraph.

Results. Hog and human renin. Intravenous injections of hog renin 3 units/mg protein and 24 units/mg protein to hypophysectomized-nephrectomized hogs were followed by an increase in blood pressure and aldosterone secretion (Table I). Two injections of human kidney renin extracts showed a similar pattern. These results compare favorably with the results of dog renin extracts reported previously(3). Although these small doses of dog renin did not increase 17-OH corticoid secretion, larger doses usually did increase it to some extent. Likewise, the larger dose of human renin, and 2 of 3 hog renin extracts increased 17-OH corticoid secretion. Injections of 115 comparative standard units of erythropoietin preparation obtained from human urine and 7.2 comparative standard units obtained from sheep plasma did not stimulate aldosterone or 17-OH corticoid secretion and had no pressor activity.

Angiotensin analogue. Previous studies (3) have shown that infusions of 0.08 and 0.17 μg/min of angiotensin II to hypophysectomized-nephrectomized dogs stimulated aldosterone secretion but not 17-OH corticoid and increased both systolic and diastolic pressures (mean rise in blood pressure mm Hg systolic/diastolic = 23/27, $P<0.01$). In fact, the pressor response was proportional to dose from 0.04 to 1.7 μg/min. Only with the larger doses was 17-OH corticoid secretion increased. Infusions of much larger doses, 0.4 to 8.0 μg/min of the angiotensin analogue, angiotensin II-diamide, produced a small but significant increase in aldosterone secretion, although peak aldosterone levels were still within the range of the basal secretion rates observed in hypophysectomized-nephrectomized dogs. The difference between the post angiotensin and the post analogue secretion rates was highly significant, $P<.01$. These doses of the analogue increased diastolic blood pressure slightly but significantly (mean rise in blood pressure 3 mm Hg, $P<0.01$) but had no effect on systolic blood pressure. There was no dose-response relationship with respect to blood pressure or aldosterone secretion so the results from all infusions were averaged (Fig. 1). 17-OH corticoid secretion was not affected.

Carboline infusion. The results of the infusions of 1 methyl-6-methoxy-1, 2, 3, 4, tetrahydro-2-carboline in hypophysectomized and in hypophysectomized-nephrectomized dogs are summarized in Fig. 2. None of the

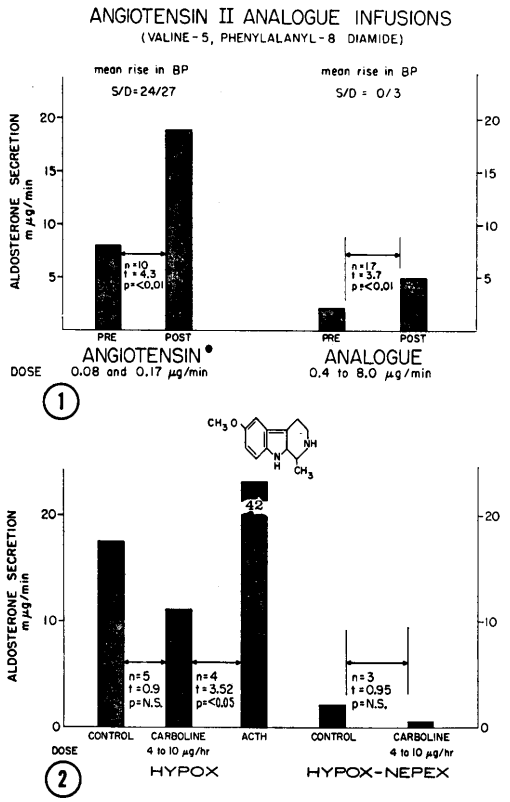

FIG. 1. Effect of infusions of angiotensin II or valine-5-phenylananyl-8-diamide upon aldosterone secretion in hypophysectomized-nephrectomized dogs.

FIG. 2. Effect of infusion of 1-methyl-6-methoxy 1, 2, 3, 4, tetrahydro-2-carboline upon aldosterone secretion in hypophysectomized and hypophysectomized-nephrectomized dogs.

* Angiotensin II data have been reported(3).

doses of carboline had any stimulatory effect upon aldosterone or 17-OH corticoid secretion, and there was no difference between the infusions of carboline in 10% ethanol in saline or 0.1% ascorbic acid in saline. Consequently, the control secretion rates and those during infusion were averaged. The bars represent the means of aldosterone measurements in 2 samples of adrenal vein blood during control periods for each dog and 3 to 4 samples collected at one-half hour intervals during the carboline infusions. The ACTH bar represents the mean of a single measurement in each of 4 dogs. There was no effect upon aldosterone secretion in the 5 hypophysectomized and 3 hypophysectomized-nephrectomized dogs by the carboline infusions. Blood pressure also did not change. As shown previously, nephrectomy results in a marked decrease in the control aldosterone secretion(3). These low values persisted despite the carboline infusion, yet in the hypophysectomized-nephrectomized dog, renin, angiotensin II and ACTH administration increased aldosterone secretion.

Discussion. Previous studies have shown that renin extracts of dog kidneys stimulate aldosterone secretion in hypophysectomized-nephrectomized dogs(1-6). The present study demonstrates that with further purification of a hog renin extract aldosterone stimulatory activity is retained and remains with the pressor activity and that human renin can also stimulate aldosterone secretion.

The experiments with the angiotensin II analogue demonstrate that only a slight change in the angiotensin II molecule markedly diminishes aldosterone stimulation and pressor response. This is additional evidence for the specificity of the aldosterone stimulating effect of angiotensin. Previous reviews (10,11) have pointed out the structure-pressor relationships. This is the first time that the aldosterone stimulating effect has also been altered by decreasing the pressor effect. This does not mean that the stimulation of aldosterone by angiotensin II is due to a rise in blood pressure, since doses of norepinephrine which markedly increase blood pressure do not increase aldosterone secretion (3). Rather it suggests that the biological activities of angiotensin, *i.e.*, the smooth muscle contraction and aldosterone stimulation are dependent upon the same functional groups in the angiotensin molecule.

The failure of 1 methyl-6-methoxy-1, 2, 3, 4, tetrahydro-2-carboline to stimulate aldosterone secretion in present experiments is in contrast to the results of Farrell *et al.*(8). These workers claimed that doses of carboline even smaller than used in the present experiments stimulated aldosterone secretion in midcollicular decerebrate dogs. The present experiments were undertaken to determine whether carboline acted by stimulating renin secretion but it did not stimulate aldosterone secretion in hypophysectomized dogs with intact kidneys and intact central nervous sys-

tem. Recently, Taylor *et al.*(12) have reported that the effect of carboline infusions upon aldosterone secretion in normal dogs was inconsistent. The failure of carboline to stimulate aldosterone secretion in simple hypophysectomized dogs, a preparation which responds to hemorrhage, salt depletion, inferior vena cava constriction, aortic constriction above the renal arteries, ACTH, renin and angiotensin with an increase in aldosterone secretion(2,3,4,5,6) casts doubt upon the role of this carboline in regulation of aldosterone secretion.

Summary. Injections of hog renin extracts with a purity of 24 units/mg protein and semipurified extracts of human kidney increased aldosterone secretion in hypophysectomized-nephrectomized dogs. Two erythropoietin preparations were inactive. Infusions of large doses of the angiotensin analogue (5-valine, 8-phenylalanine diamide) produced a minimal pressor response and only a slight increase in aldosterone secretion. Infusions of 1-methyl-6 methoxy-1, 2, 3, 4, tetrahydro-2-carboline into simple hypophysectomized or hypophysectomized-nephrectomized dogs did not stimulate aldosterone secretion.

The authors wish to thank Mr. Roy Shackelford, Mrs. Annette Lowe and Mr. Walter Hirniak for technical assistance. The angiotensin analogue was kindly supplied by Dr. Robert Schwyzer, Ciba Foundation, Basle, Switzerland, 1-methyl, 6-methoxy, 1, 2, 3, 4, tetrahydro-2 carboline by Dr. Gordon Farrell, hog renin by Drs. Weisenbaugh and S. W. Hoobler and erythropoietin preparations by Dr. D. C. Van Dyke.

1. Ganong, W. F., Mulrow, P. J., *Nature,* 1961, v190, 115.

2. Mulrow, P. J., Ganong, W. F., *Yale J. Biol. & Med.,* 1961, v33, 386.

3. Mulrow, P. J., Ganong, W. F., Cera, G., Kuljian, A., *J. Clin. Invest.,* 1962, v41, 505.

4. Carpenter, C. C. J., Davis, J. O., Ayers, C. R., *Fed. Proc.,* 1961, v20, 178.

5. Davis, J. O., Carpenter, C. C. J., Ayers, C. R., Holman, J. E., Bahn, R. C., *J. Clin. Invest.,* 1961, v40, 684.

6. Davis, J. O., Ayers, C. R., Carpenter, C. C. J., *ibid.,* 1961, v40, 1466.

7. Bartter, F. C., Casper, A. G. T., Delea, C. S., Slater, J. D. H., *Metabolism,* 1961, v10, 1006.

8. Farrell, G., McIsaac, W. M., *Arch. Biochem. & Biophys.,* 1961, v94, 543.

9. Keighley, G., Lowy, P. H., Borsook, H., Goldwasser, E., Gordon, A. S., Prentice, T. C., Rambach, W. A., Stohlman, F., Van Dyke, D. C., *Blood,* 1960, v16, 1424.

10. Page, I. H., Bumpus, F. M., *Physiol. Rev.,* 1961, v41, 331.

11. Gross, F., Turrian, H., *Polypeptides Which Effect Smooth Muscles and Blood Vessels.* Edited by M. Schachter. 1959, p137.

12. Taylor, A. N., McIsaac, W. M., Farrell, G., *Fed. Proc.,* 1962, v21, 186.

Received October 3, 1962. P.S.E.B.M., 1963, v112.

Interference by Newborn Lymphoid Cells with Establishment of Immunologic Unresponsiveness to a Protein Antigen.* (27934)

M. W. COHEN AND G. J. THORBECKE† (Introduced by B. Benacerraf)
Department of Pathology, New York University School of Medicine, New York City

Immunologic unresponsiveness to a protein antigen may be established at any time during the life of an animal, although with less facility as the animal matures. Smith *et al.*(1,2) quantitated the phenomenon in newborn and immature rabbits with bovine serum albumin and established a relationship between dosage and duration of the resulting unresponsiveness. While milligram amounts suffice in neonatal animals, others(3,4) have shown that several hundred times as much protein are required in adult animals.

Some investigators have suggested that the induction of tolerance during the neonatal period depends upon a certain immaturity of the lymphoid cells unique to this

* Supported by grant from U.S.P.H.S.

† Recipient of career development award.