1960

Action and regulation of secretion of aldosterone

Jack E. Talsma
University of Nebraska Medical Center

ACTION AND REGULATION OF SECRETION OF ALDOSTERONE

SENIOR THESIS

UNIVERSITY OF NEBRASKA

COLLEGE OF MEDICINE

JACK E. TALSMA

1960

HISTORY

The study of the hormones of the adrenal cortex dates from 1930 when Swingle and Pfiffner[1], and Hartman and Brownell[2] first prepared adrenal extracts used as substitution therapies in the absence of endogenous adrenal function. Then in 1934 Wintersteiner, Vars, and Pfiffner[3,4], after having removed various crystalline substances from adrenal extracts noticed that there remained an amorphous, non-crystalline substance which was considerably more active than the crystalline extracts. Related studies by Cartland, Kurzinger, and associates[5,6], showed high activity in the amorphous fraction which could not be attributed to desoxycorticosterone or other known corticoids.

Most of the biologic work with the early amorphous fraction was carried out with the preparation of Kendall, Mason, and associates. They discovered that the amorphous fraction contained about one-half the life maintaining activity found in whole adrenal extracts. However, this fraction did not inhibit growth or cause adrenal atrophy as effectively as the other steroids[7] and its action on carbohydrate metab-

olism was weak. Although the preparation used was not pure, the cumulative implication of the findings was that the amorphous fraction had a wider range of biologic actively than desoxycorticosterone and in certain respects a higher potentcy than any known corticoid.

In 1952, Simpson and Tait[8] began to publish the results of a systematic re-investigation of the activity of the amorphous fraction of adrenal extract. Using improved methods of clinical investigation they again showed the potency of adrenal extracts in affecting sodium and potassium excretion was greatly in excess of what could be accounted for by any known adrenal steroid or combination of steroids[8,9,10]. They also showed that this mineralocorticoid activity was also present in adrenal venous blood[11] thus adding strength to the assumption that a true hormone was involved.

An all out effort was made towards the isolation of this hormone and in 1953, Simpson, Tait, and Wett-stein, Neher, and von Euw, Reichstein [12] isolated and crystallized aldosterone. Shortly thereafter other workers isolated the steroid and a substance was purified from urine which later proved to be aldosterone.

BIOCHEMISTRY

To understand the chemical nature of aldosterone, it is best to relate its biological activity to structural modifications from the best known of the adrenocortical steroids. In Figure 1 is shown the basic ring structure or the parent hydrocarbon of all the steroid hormones. It is a completely saturated hydrocarbon consisting of four rings. All the valences of carbon not satisfied by linkage toad jacent carbon atoms are taken up by hydrogen atoms, even though the hydrogen is not shown. The carbon atoms are numbered according to the diagram.

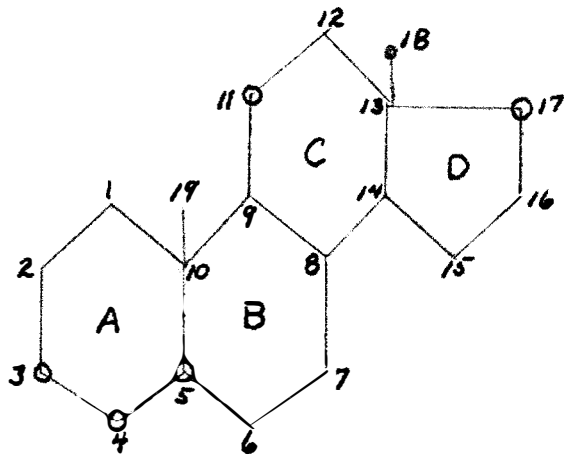


Figure 1
PARENT HYDROCARBON OF STEROID HORMONES

The carbon atoms indicated by open circles are the ones that are modified in one or more of the more familiar of biologically active cortical hormones. These modifications endow the different molecules with various biological activities and potencies. Carbon 18 is the position at which aldosterone alone among the known naturally occurring steroid hormones is uniquely substituted.

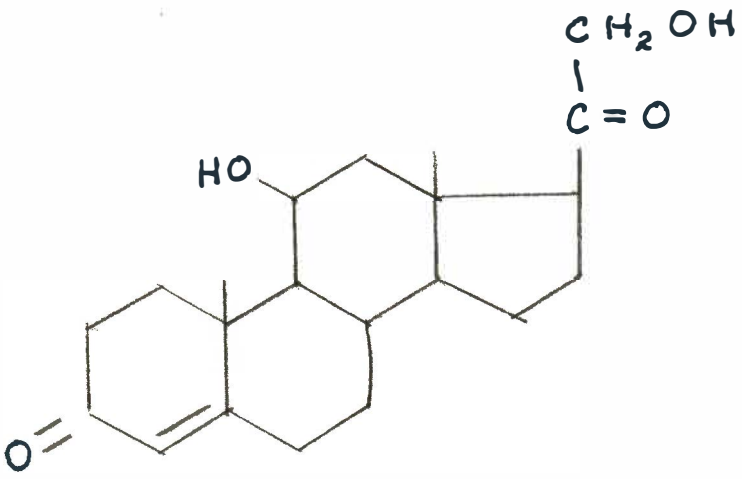


Figure 2
CORTICOSTERONE

In Figure 2 is diagrammed the molecular structure of corticosterone a typical cortisteroid. It differs from the parent hydrocarbon at all of the five carbons

which were indicated on the first diagram by open circles. In rings A and B a ketone group replaces the two hydrogens on carbon 3 and a double bond forms between carbon 4 and carbon 5. This structural modification occurs between all active corticosteroids. Corticosterone is also typical in having a 2-carbon side chain in place of one hydrogen atom at carbon 17. The carbons of this side chain both have oxygen functions consisting of an hydroxyl and a ketone group. Corticosterone is a fairly potent glucocorticoid, and the substituent of the molecule which specially endows it with glucocorticoid activity is the oxygen function, present as an hydroxyl group, at carbon 11. All compounds with distinct glucocorticoid activity have an oxygen group at carbon 11, either as an hydroxyl or a ketone.

Glucoticorticoid activity is intensified if in addition to an oxygen at carbon 11 an hydroxyl group replaces the remaining hydrogen at carbon 17 as is shown in the structure for cortisol, which is identical otherwise to corticosterone. Cortisone, more potent than corticosterone but less potent than cortisol, differs from cortisol only in that the oxygen at

carbon 11 is present as a ketone rather than as an hydroxyl group. Therefore small differences in the oxygen functions can be seen to be responsible for great differences on potency of glucocorticoids.

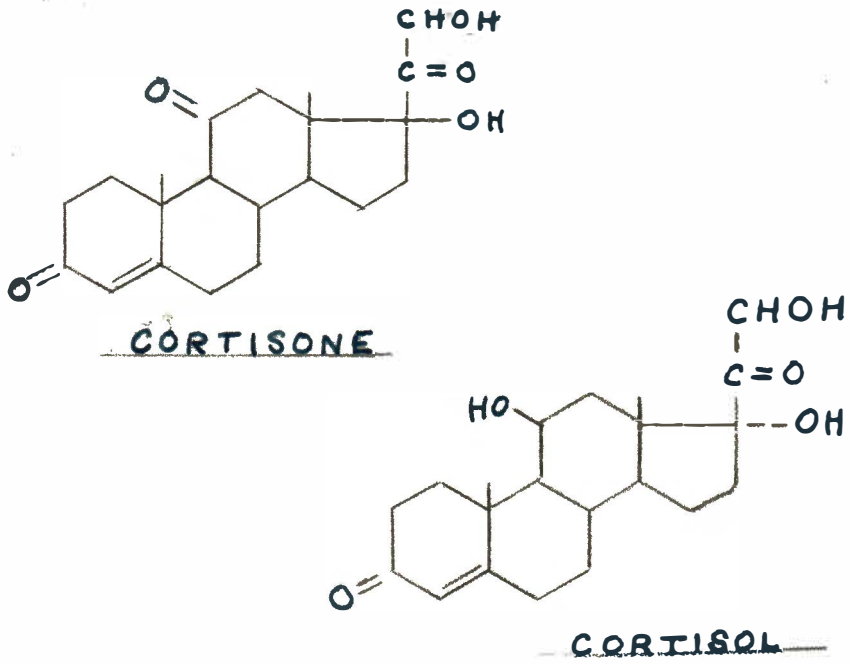
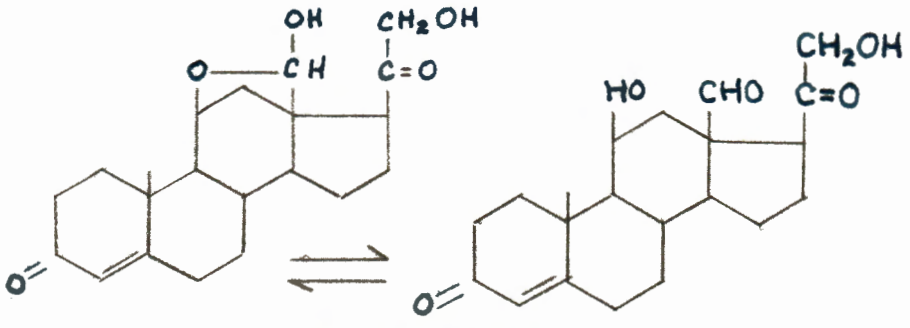


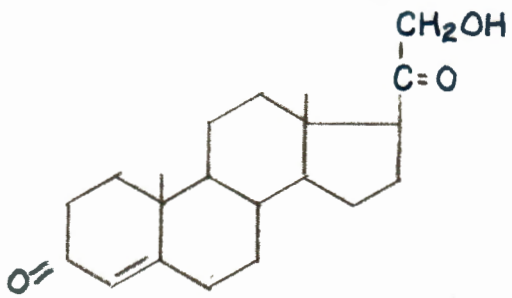
Figure 3
GLUCOCORTICOIDS

In Figure 4 are shown the two most potent mineralocorticoids, aldosterone and desoxycorticosterone. As the name indicates desoxycorticosterone has an absence of the hydroxyl group at carbon 11. Since it is also without an hydroxyl at carbon 17, it can be predicted from our knowledge of the relationship of structure to function that this compound is practically

devoid of glucocorticoid activity.



ALDOSTERONE



11 - DESOXYCORTICOSTERONE

Figure 4
MINERALOCORTICOIDS

With the preceding analysis of corticosteroid structure in mind, it is now possible to examine the aldosterone molecule. Aldosterone in its crystalline form has an aldehyde group at carbon 18, hence its name. In solution it exists as an equilibrium between the hemiacetal form. In referring to the crystalline aldehyde form it is noted that aldosterone is identical

to corticosterone except for modification at one additional carbon atom. One would therefore expect to have glucocorticoid activity equal to that of corticosterone and in this respect to be different from desoxycorticosterone. Its unique modification, the aldehyde group at carbon 18, must be assumed to be the structural feature which endows this compound with its extremely high potency in the regulation of electrolyte metaboliam.

BIOLOGICAL ACTION

For convenience the adrenal steroids have been categorized as either mineralocorticoids or glucocorticoids. However this is not entirely correct since each compound, even though predominantly of one activity, also possesses the other to a minor degree.

From its structural formula it can be predicted that aldosterone will possess glucocoticoid activity by virtue of the hydroxyl group on carbon 11. Its potency in regulating electrolyte metabolism is shown by each of the four pertinent tests to be greater than that of desoxycorticosterone. In table I it is seen that the relative potencies of these two compounds are not the same indicating subtle differences between them in their physiological activity.

TABLE I.: BIOLOGICAL ACTIVITIES OF CRYSTALLINE ALDOSTERONE

	Potency of Aldosterone Relative to:	
	Desoxycorticosterone	Cortisone
For mineralocorticoid activity		
Sodium Retnetion	25x	-
Potassium Excretion	5x	-
Na/K Excretion	120x	-
Maintenance Adx. Dog	25-30x	-
For glucocorticoid activity		
Cold Stress	/	= or /
Liver Glycogen Deposition	30x	1/3
Eosinophil Depletion	-	1/2 - 1/4
ACTH Suppression	-	1/2 - 1/3

The glucocorticoid effects of aldosterone, although not significant in the amounts secreted by the normal gland, have been studied by various investigaters. Gaunt[13], in experiments on adrenalectomized rats showed that aldosterone was as active or more active than cortisone in its protection against the stress of cold, and considerably more active than desoxycorticosterone. Also Soffer[14] reported that an Addisonian patient maintained on 100 micrograms of aldosterone per day withstood the stress of an upper respiratory infection.

In adrenal insufficiency there is characteristerically a marked susceptibility to the stress of water intoxication due to an inability to excrete excess water and to a lack of resistance to the toxic effects of

water. Equally effective protection against the experimental production of this syndrome in rats has been shown by aldosterone and hydrocortisone.

In relation to carbohydrate metabolism aldosterone has caused a deposition of liver glycogen in adrenalectomized fasting mice[15]. Its potency was about one third that of cortisone and thirty times that of desoxycoticosterone. However the effects of aldosterone on protein and carbohydrate metabolism have not been consistent and those who have made the most extensive studies have failed to see any significant effects.

The effects of aldosterone on blood pressure have been studied fairly extensively. Although it will maintain a normal blood pressure in adrenalectomized animals and Addisonian patients, no direct evidence exists that it will produce hypertension in the manner of desoxycorticoterone and the other steroids. Gross[16] ran extensive experiments comparing the effects of aldosterone and desoxycorticosterone on adrenalectomized rats given a 1 per cent solution of sodium chloride to drink. Desoxycorticosterone given daily to these animals produced hypertension, vascular and renal lesions and a diabetes insipidus-like water exchange. When aldosterone was

given in one twenty-fifth the dose it showed none of the pathologic effects produced by desoxycorticosterone.

The amount of aldosterone secreted by the normal adrenal though, is so minute that its glucocorticoid activity can be considered to be negligible. The normal level of secretion of aldosterone is roughly 100 times less than that of hydrocortisone whereas equal quantities of these hormones exhibit similar glucocorticoid properties.

The primary effect of aldosterone is its role in the regulation of sodium retention and potassium excretion by the kidney. The concept of Loeb[17] that the adrenal steroids controlled extracellular sodium by regulating the renal excretion of sodium has been challenged by Swingle[18], Overman[19], and Wilson[20]. Even though aldosterone has been shown to be a potent salt retaining hormone, a direct demonstration of the renal tubular effect was not performed until Barger and Berlin[21] reported their infusion experiments on dogs. In their experiment they catherized unilaterally the renal artery in four dogs, and then catherized each kidney individually so as to collect the urine separately from each kidney. This method enabled them to infuse aldos-

terone and other steroids into one kidney and compare the results of electrolyte excretion with the non-affected kidney of the other side as a control. They ran the experiments on normal dogs and on adrenalectomized dogs.

In the normal dogs on an isotonic saline diuresis the intrarenal infusion of aldosterone over a wide dosage range did not produce a detectable decrease in sodium excretion from the injected kidney as compared to the non-infused kidney. However in all dosages used there was demonstrated a marked excretion of potassium. This kaluresis was limited to the infused side thus indicating a direct renal effect of the steroids on potassium excretion, but no detectable effect on sodium excretion in the normal animal.

It was interesting to note that after adrenalectomy a unilateral renal antinatriuretic effect of the steroids as well as a kaluretic effect could be demonstrated. The effects on potassium excretion were quantitatively equal in the normal and the adrenalectomized dog. Thus aldosterone produced a kaluresis which was similar in the normal dog. This suggests the possibility that there might be a direct renal antagonism between aldosterone and the other adrenal steroids secreted from the adrenal cortex.

The separation of the action of aldosterone on sodium and potassium excretion in the normal dog, and the greater effect on sodium excretion as compared to potassium excretion in the adrenalectomized dog, do not support the hypothesis of a simple cation exchange of sodium and potassium in the distal tubule, but rather to an independence of movement. These observations on the direct renal effect of the steroids on potassium and the lack of effect on sodium on the n rmal animal may explain the kaluresis seen in primary hyperaldosteronism, and the absence of edema formation.

Aldosterone appears to affect tubular function in the kidney, but the precise site and mode of action have not been elucidated. Both the proximal and distal rubules are implicated. In the distal tubule a cation exchange mechanism appears to be involved as described by Pitts and Alexander[22] and Berliner, Kennedy and Orloff[23]. Bartter[24] and Crabbe have shown increased potassium and hydrogen ion excretion that is parallel but not quantitatively identical to increased sodium retention and with a variable potassium ratio. However the experiments of Barger and Berlin, described earlier, demonstrate that sodium retention and potassium excretion

cannot be explained on a simple cation exchange.

Aldosterone may act before the region of the distal tubular cation exchange and perhaps on the proximal tubule. Nicholson[25] found that the hormone did not cause sodium retention after the renal proximal tubule had been damaged by 0.5 percent racemic sodium tartrate injected into the renal artery, whereas sodium retention did occur after the distal tubule had been damaged by 0.005 per cent mercuric chloride instilled via the ureter. With a low-sodium diet or in cases of congestive heart failure or portal cirrhosis exogenous aldosterone will cause almost complete soidum reabsorption and will not increase potassium excretion. Thus it appears that sodium is not retained exclusively by cation exchange but is reabsorbed at a more proximal point in the kidney tubule.[26]

REGULATION OF ALDOSTERONE SECRETION

PITUITARY AND ACTH

Although the mechanisms which regulate the secretion of aldosterone are still debatable, one important fact is all but certain; aldosterone is not under the complete and direct control of ACTH, as are the other major corticoids.

This is apparent from the fact that life and electrolyte balance can be maintained after hypophysectomy and that hypophysectomized animals die promptly after adrenal adrenalectomy, that the adrenal must secrete some DC-like material independent of the pituitary. Also many investigators have found that ACTH administration in man does not significantly increase the blood or urine levels of aldosterone under conditions which cause an increased production of other corticoids.

It has been demonstrated that after hypophysectomy in the dog, aldosterone secretion continued in measurable amounts-not enhanced by ACTH.[2] In fact aldosterone was the only corticoid present after hypophysectomy in amounts sufficient to exert important metabolic effects. This is entirely consistent with the observation that panhypopituitary patients excrete normal amounts of aldosterone although the secretion of other corticoids is greatly reduced.

ELECTROLYTES

It has been demonstrated repeatedly and well substantiated that dietary electrolytes changes affect aldosterone secretion. Increased aldosterone secretion was found consistently with sodium restriction but an

otherwise normal diet.[24,32] Simultaneous potassium restriction inhibited[33] or reduced[32] this response to sodium restriction. Potassium loading in the presence of a low-sodium diet resulted in increased aldosterone values.[24,32, 33] Sodium loading was shown to decrease aldosterone excretion in normal subjects.[32] Potassium restriction in the normal subject has been shown to decrease aldosterone secretion.[34]

Reduction of sodium intake appears to be the most important stimulus to increased aldosterone output. A large number of clinical investigations support this fact. However sodium deprivation cannot be the only stimulus to increased aldosterone decretion. Marked changes in the aldosterone secretion rate can occur during blood loss without change in serum sodium. Reduction of body fluid volume by dehydration or diuretics has been reported to increase the urinary level of the steroid without changes in electrolytes. This has led certain workers to postulate that the effect of sodium deprivation is due to contraction of body fluid volumes acting presumably on volume receptors. However, the effects of changes in body water are best seen when the subject is depleted of sodium beforehand, and there is

some question whether there is a physiologically significant change in urinary aldosterone in the dehydrated normal subject in the absence of prior sodium depletion.

Since sodium is one of the main stimuli for aldosterone secretion, the question arises as to how the organism is able to detect changes in the sodium content of its body fluids and to adjust aldosterone output accordingly. Electrolyte receptors may exist in the vascular system or brain stem, similar to chemoreceptors and taste buds. However there has been no extensive investigation or explorationinto this field.

The role of potassium in the regulation of aldosterone secretion is not definitely settled. Potassium administration to various experimental amimals has generally been shown to increase the rate of aldosterone secretion, and potassium deprivation tends to decrease the rate of secretion. However, other experiments have failed to show any significant effect of potassium administration on the aldosterone secretion rate in the adrenal venous blood in dogs.[30] Also the effects of potassium administration on urinary sodium and water excretion in man are not in accord with the concept that aldosterone secretion is increased. If the circulating

levels of aldosterone were elevated by giving potassium the expected result would be sodium and water retention. In practice, the opposite is the case; water loss and natriuresis are the rule.[31,40]

One explanation for the discrepancy between the results of potassium administration has been suggested.[41] Potassium may increase renal loss of aldosterone by decreasing tubular reabsorption, thus lowering the circulating level of the steroid while increasing the level in the urine.

Although the effects of these dietary changes on aldosterone are accepted as fact, their method of action is still uncertain. One of the more popular theories is that the effects occur through volume changes. The aldosterone response to changes in body fluid volume appears to take precedence over changes in electrolyte intake and concentration under certain conditions.

VOLUME

Liddle[35] first reported a decrease in urinary sodium-retaining factor when the body fluids of sodium-depleted subjects and patients with edema were expanded with vasopressin and water or intravenous administration of physiologic saline solution. Subsequent experiments

have confirmed these findings. However there are conflicting results as to whether the increase in sodium excretion results from a decrease in aldosterone secretion; since sodium excretion occurs during expansion of fluid volume despite continued administration of large amounts of aldosterone.[36] Bartter[37] further demonstrated that aldosterone excretion decreased with increased extracellular was increased, decreased or unmodified: increased aldosterone excretion occurred when extracellular fluid volume was decreased, regardless of whether intracellular water or serum sodium concentration rose or remained unchanged. More recently he concluded that the regulation of aldosterone secretion by changes in extracellular fluid volume ultimately depends upon a function of intravascular volume. In experiments on normal subjects and patients with hypoproteinemia, intravascular expansion by salt-poor albumin and contraction by phlebotomy was associated with the anticipated change in aldosterone excretion.

However, there is evidence that aldosterone secretion is not solely a function of intravascular fluid volume. Elevated aldosterone secretion may occur in congestive heart failure despite increased plasma volumes.[38]

Farrell et al[39] observed a marked increase in adrenal venous-blood aldosterone in response to blood loss that was only partially prevented by infusion of plasma substitutes and could not be correlated with changed in serum electrolytes or total blood volume.

In experiments on dehydration in a normal subject on a normal sodium intake the changes in urinary aldosterone were quite modest and of questionable significance.[42] The observed changes were within the limits of normal day-to-day variation in urinary aldosterone, and were inconsequential when compared to the increase on urinary aldosterone induced by low sodium intake. Muller failed to find a significant effect on urinary aldosterone from simple loss of body water, unless accompanied by sweating and physical excercise. Futhermore marked changes in aldosterone output in the sodium-depleted individual has been seen to occur without significant changes in the volume of the body fluids.[43]

Thus there is still lacking a convincing demonstration that aldosterone secretion is entirely regulated by changes in some particular fluid-volume compartment, but there is evidence that volume changes do effect the output to a considerable degree.

MISCELLANEOUS EFFECTS

Recent studies on dogs indicate that the degree of distention of the right atrium may play a role in controlling aldosterone output. When the right atrium is stretched by external sututrs the aldosterone secretion is reduced to one-half that in controls.[47] Stretch on the left atrium had no effect. This finding suggests a reflex system affecting aldosterone secretion, with receptors located in the right atrium.

Emotional stimuli such as excitement and anxiety have been shown to increase aldosterone output.[48] This finding suggests that descending pathways from higher centers may modify the activity of the brain stem areas which may be concerned with aldosterone regulation. This raises the question as to the validity of experiments on aldosterone secretion involving pain or trauma and there resultant emotional stimuli.

HUMORAL FACTORS

There is suggestive evidence that the secretion of aldosterone is controlled by a trophic factor. Removal of the head of the experimental animal with the trunk maintained under normal conditions of blood pressure body temperture, and oxygenation, results in a profound fall in the aldosterone secretory rate. If the carotid arteries and jugular veins are left intact, the dissection otherwise the same as in complete decapitation, the output of the steroid is normal.[44] This speaks strongly for a humoral agent arising in the head.

Farrell and associates have carried out experiments that support the concept of a cephalic structure involved in the regulation of aldosterone secretion.[45] Removal of the head results in a marked fall in the aldosterone secretion rate, but section of nervous connections are intact. Also removal of the brain substance rostral to the corpora quadrigemina resulted in a profound fall in the output of the steroid. From their experiments they arrived at two conclusings: 1) the secretion of aldosterone is controlled by a cerebral structure, probably in the diencephalon, and 2) the mode of regulation is humoral, involving a trophic

hormone.[46] In the cat, lesions of the central core of the midbrain and caudal diencephalon are quite effective in reducing the secretion of aldosterone.

From these experiments Farrell propsed a hypothesis with regard to the central regulations of aldosterone secretion. He theorized that a caudal diencephlic or anterior midbrain center controls the secretion of a humoral tripic factor which stimulates aldosterone secretion by the zona glomerulosa. The center receives stimulatory or inhibitory impulses from peripheral receptors, and is influenced by other parts of the brain stem. This center may be directly effected by such factors as blood flow, and plasma electrolyte or hydrogen ion concentrations.

CONCLUSION

The final understanding of the physiological mechanism by which the secretion of aldosterone is controlled must await the identification of receptors, afferent pathways and central integration. It is unlikely that there is any one stimulus to aldosterone secretion, but it is probably a complex regulatory system analogous to that for respiration. It seems feasible

that a regulatory center exists in the brain stem, which responds primarily to the level of serum electrolytes, acting directly or by way of chemoreceptors. Its activity can also be altered by higher centers such as emotion and anxiety. There probably exists stretch receptors located in the right atrium and, possibly, the great veins which respond to changes in the volume of body water as reflected in the volume of the vascular system. From the numerous experiments it seems that under most circumstances, the sensitivity of the aldosterone-regulating mechanism to sodium ions is greater than to changes in volume, so that the latter play a minor role in controlling aldosterone secretion. However, under extreme reduction in body water, the volume receptors become of importance. Futher experiments still are needed to substantiate the theory of central regulatory mechanism.

BIBLIOGRAPHY

1. Swingle, W.W., and Pfiffner, J.J.: Science 71:321, 1930.

2. Hartman, F.A. and Brownell, K.A.: Science 72:76, 1930.

3. Pfiffner, J.J., Wintersteiner, O., and Vars, H.M.: Biological Chemistry 111:585, 1935.

4. Wintersteiner, O., Vars, H.M., and Pfiffner, J.J.: Journal of Biological Chemistry 105: C, 1934.

5. Cartland, G.F., and Kuizenga, M.H.: Endocrinology 24:526, 1939.

6. Kuizenga, M.H.: American Association of Advanced Science 1944, P. 57.

7. Wells, B.B., and Kendall, E.C.: Proc. Staff Meeting Mayo Clinic, 15:133, 1940.

8. Grundy, H.M. and Simpson, S.A., and Tait, J.F.: Nature 169:795, 1952.

9. Grundy, H.M., Simpson, S.A., Tait, J.F.: Acta Endocrinology 11:199, 1952.

10. Tait, J.F., Simpson, S.A., and Grundy, H.M.: Lancetl: 112, 1952

11. Simpson, S.A., Tait, J.F., Wettstein, A., Neher, R., von Euw, J., and Reichstein, T.: Experientia 10:132, 1954.

12. Simpson, S.A., Tait, J.F., Bush, I.E.: Lancet 2:226, 1952.

13. Gaunt, R., Gordon, A.S., Renzi, A.A.: Endocrinology 55:236, 1954.

14. Soffer, L.J., Griboff, S., Weiner, R., Eisenberg, J., Ianaccone, A.; Metabolism, 1955

1 5. Schuler, W., Desaulles, P., and Meier, R.: Experienten 11:67, 1955

16. Gross, F., Loustalot, P., and Meier, R.: Experientia 10:142, 1954.

17. Loeb, R.F., Atchley, D.W., Benedict, F.M., and Leland, J.: Journal of Experimental Medicine 57:775, 1933.

18. Swingle, W.W., Pfiffner, J.J., Vars, H.M. and Parkins, W.M.: American Journal of Physiology 108:144&428, 1934.

19. Overman, R.R., Flanagan, J.B., and Davis, A.K.: American Journal of Physiology 160:104, 1957.

20. Wilson, D.L.: American Journal of Physiology 190:104, 1957.

21. Barger, A.C., Berlin, R.D., and Tulenko, J.F.: Endocrinology 62:804, 1958.

22. Pitts, R.F., and Alexander, R.S.: American Journal of Physiology 144:239, 1945.

23. Berliner, R.W., Kennedy, T.J., and Orloff, J.: American Journal of Medicine 11:274, 1951.

24. Bartter, F.C.: Metabolism 5:369, 1956

25. Nicholson, T.F., Canad, J.: Biochemistry Physiology 35:641, 1957.

26. Nelson, D.H., and Aufust, J.T.: Journal of Clinical Investigation, 1958.

27. Farrell, G.L., Royce, P.C., Rauschkolb, E.W., and Hirschmann, H.: Proc. Soc. Experimental Biology and Medicine 87:141, 1954.

28. Luetscher, J.A., Deming, Q.B., and Johnson, B.B.: Ciba Foundation Colloquium Endocrinology 4:530, 1952.

29. Farrell, G.L., Banks, R.C. and Koletsky S.: Endocrinology 58: 104, 1956.

30. Rosnagle, R.S. and Farrell, G.L.: American Journal of Physicology 187:7, 1956.

31. Falbriard, A., Muller, A.F., Neher, R., and Mach, R.S.: Schweiz Medical Journal 85:1218, 1955.

32. Johnson, B.B., Liebernam, A.H. and Mulrow, P.J.; Journal of Clinical Investigation 36:757, 1957

33. Laragh, J.H. and Stoerk, H.C.: Journal of Clinical Investigation 36:383, 1957.

34. Singer, B., and Stack-Dunne, M.P.: Journal of Endocrinology 12:130, 1955.

35. Liddle, G.W., Bartter, F.C., and Thorn, G.W.: Journal of Clinical Investigation, 1958.

36. Agust, J.T., Nelson, D.H., and Duncan, L.E.: Journal of Clinical Investigation 34:949, 1955.

37. Bartter, F.C., Liddle, G.W., Duncan, L.E., Barber, J.K.: Journal of Clinical Investigation 35: 1306, 1956.

38. Luft, R., Sjogren, B., Ikkos, D.: Recent Program on Hormone Research 10:425, 1954.

39. Farrell, G.L., Rosnagle, R.S., and Rauschkolb, E.W.: Circulation Research 4:606, 1956.

40. Liddle, G.W., Bennett, L.L. and Forsham, P.: Journal of Clinical Investicaltion 32:1197, 1953.

41. Rosnagle, R.S. and Farrell, G.L.: American Journal of Physiology 187:7, 1956.

42. Bartter, F.C., Liddle, G.W., Duncan, L.E. and Barber, J.K.: Journal of Clinical Investigation 35:757, 1957.

43. Johnson, B.B., Liebermann, A.J. and Mulrow, P.J.: Journal of Clinical Investigation 35:1306, 1956.

44. Rauskolb, E.W., and Farrell, G.L. : Endocrinology 59:526, 1956.

45. Rauskolb, E.W., Farrell, G.L. and Koletsky S.: American Journal of Physicology 182:269, 1955

46. Farrell, G.L., Rauskolb, E.W. and Royce, P.C.: American Journal of Physiology 182: 269, 1955.

47. McCally, M., Anderson, C.H., and Farrell, G.L.: Proc. 40th Mtg. Endocrinology Society 1958 P.119.

48. Venning, E.H., Dyrenfurth, I., and Beck, J.C.: Journal of Clinical Endocrinology & Metabolism 17:1005, 1957.