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A COMPARISON OF THE DIRECT RENAL ACTIONS OF PITUITARY GROWTH AND LACTOGENIC HORMONES

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The growth hormones from man, monkeys, whales, ox, sheep and pig differ in molecular weight, isoelectric pH, the number of —S—S— bridges per molecule, N- and C-terminal sequences and antigenic properties (Wilhelmi, 1960; Li, 1960). There are also differences in the electrophoretic behaviour of these molecules (Ferguson & Wallace, 1961). The electrophoretic similarity of the growth and lactogenic hormones (Ferguson & Wallace, 1961; Ferguson, 1964) is confined to the hormones of man (Ferguson, 1964). Lockett & Nail (1965) were, however, unable to differentiate the urinary changes induced in rats by single injections of bovine and ovine lactogenic and growth hormones, despite the separate chemical identities of these molecules.

The actions of bovine growth hormone on the isolated kidney have been the subject of a previous paper (Lockett & Roberts, 1963). A more detailed comparison of the direct renal actions of human, bovine and ovine growth hormones, and of the bovine and lactogenic hormones, has now been made since a true similarity between the actions of these large polypeptides would be suggestive of an amino acid sequence common to them all.

METHODS

Heart-lung-kidney preparations were made in spinal cats as previously described (Davey & Lockett, 1960). The blood used to fill the perfusion circuit was collected from male, female or neutered spinal animals and contained 50 mg creatinine hydrochloride (British Drug Houses Ltd.) and 1000 units of heparin (Evans Medical Ltd.) per 100 ml. Thirty-one preparations have been used predominantly for the study of the bovine and seven for the study of the human growth hormone. Eighteen further preparations have been used for examination of the actions of ovine growth and lactogenic hormones and another three for comparison of the effects of bovine growth and lactogenic hormones. The actions of bovine growth hormone as shown by the first twenty-two experiments have been reported (Lockett & Roberts, 1963). Renal venous blood flow was measured throughout by cylinder and stop-watch.

Hormones. Highly purified ovine and bovine (Endocrine Study Group, U.S.A.), and human growth hormone (Dr K. A. Ferguson, C.S.I.R.O. Prospect, N.S.W.), and bovine (Dr K. A. Ferguson) and ovine (Dr K. A. Ferguson and Mr A. L. C. Wallace) lactogenic

hormones were dissolved at a concentration of 1 mg/ml. in 0·9 % NaCl which had been brought to pH 9·0. These solutions were stored at 7° C and were used within 10 days. Ampoules of D-aldosterone (Aldocorten, CIBA) and synthetic oxytocin (Syntocinon, Sandos Products) were diluted for use with 0·9 % NaCl.

Chemical. Concentrations of creatinine, sodium (Na) and potassium (K) in plasma and urine were determined as before (Davey & Lockett, 1960).

RESULTS

As previously reported (Lockett & Roberts, 1963) and now confirmed, physiological concentrations of bovine growth hormone (30–70 μg/150 ml. blood) reduced the rates of urinary excretion of water, Na and K, but did not change either the renal blood flow (RBF) or the creatinine clearance (GFR). An antidiuresis accompanied by a fall in the urinary concentrations of Na and K characterized the action of bovine growth hormone in blood from animals in which the intracranial circulation afforded by the vertebral arteries remained unobstructed after spinalization. Antidiuresis accompanied by a rise in the urinary concentration of Na, but

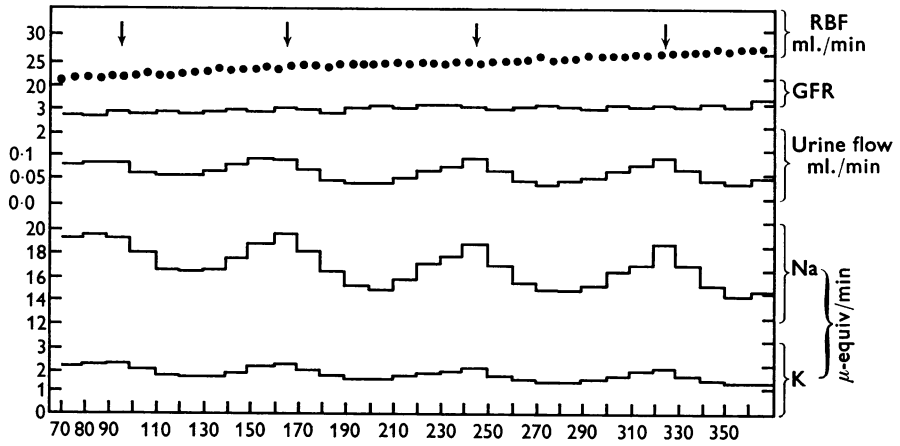

Fig. 1. Data from a kidney weighing 9·6 g perfused at 128 mm Hg and 36·5° C with blood from spinal donors. Ordinates, from above downward: renal blood flow (RBF), ml./min.; creatinine clearance (GFR); urine flow, ml./min; rates of urinary excretion, μ-equiv/min; sodium (Na) and potassium (K). Abscissae: time in minutes, from the start of the perfusion. At the first, second, third and fourth arrows the growth hormones added to the venous blood were 15 μg ovine, 30 μg bovine, 20 μg ovine and then 10 μg ovine together with 15 μg bovine, respectively. Circuit volume 150 ml.

reduction in the rate of excretion of Na, without significant change in K concentration characterized the action of the hormone in blood from animals devoid of an intracranial circulation. Ovine and human growth hormone had qualitatively similar actions on the excretion of water, Na and K by cat kidneys. Figure 1 shows that these renal actions of 20 μg

ovine growth hormone were equivalent to those of 30 μg of the bovine hormone. The action decay curves for the two hormones appeared similar for a given intensity of effect. The two hormones were additive in action. Figure 2 shows that 50 μg human growth hormone was approximately equal in intensity of action to 40 μg of the bovine hormone. The duration of the effect of the human hormone was however less than that of the bovine hormone, as has been seen throughout the work. Figure 3 demonstrates that the human and the bovine hormones are additive in direct renal action.

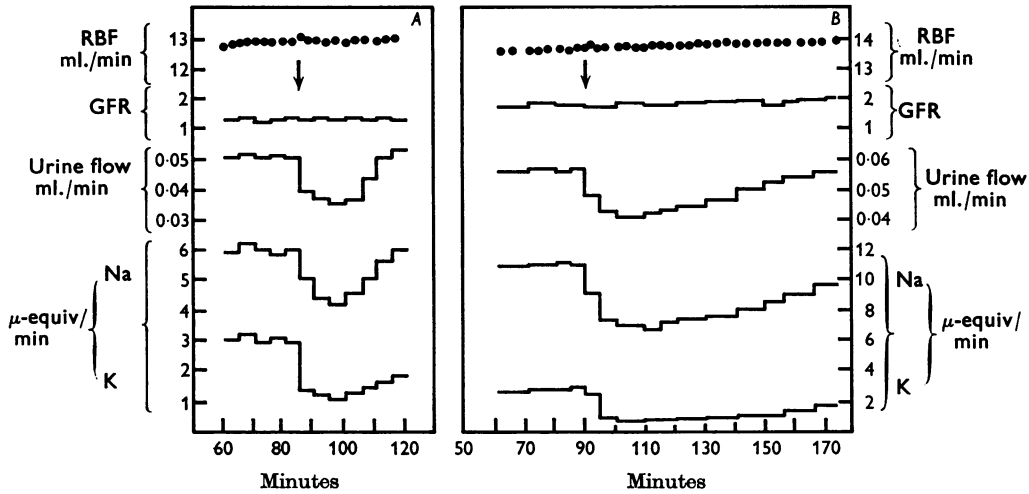

Fig. 2. Data from two kidneys *A* and *B*, weighing 5·7 and 6·2 g, perfused at 122 mm Hg and 120 mm Hg, respectively, at 36·5° C, with blood from headless donor animals. Ordinates and abscissae as in Fig. 1. At the arrows, 50 μg human growth hormone (*A*) and 40 μg bovine, growth hormone (*B*) were added to 150 ml. circuit blood.

Bovine and ovine growth hormones, as previously reported (Lockett & Roberts, 1963), converted the diuretic effect of aldosterone in blood from headless donors (Davey & Lockett, 1960) into an antidiuretic action like that of aldosterone in blood from intact animals. Figure 4 shows that when human growth hormone, 50 μg/150 ml., precedes aldosterone, the latter exerts an antidiuretic, sodium and potassium retaining action in blood from headless animals.

The lactogenic hormones, in contrast to the growth hormones, caused almost immediate and parallel increase in RBF and GFR which reached maximum in 5–7 min (Fig. 5) and thereafter returned slowly to base line. The effect of the lactogenic hormones on RBF and GFR lessened, and even disappeared (Fig. 6) when exposure to lactogenic hormone was

repeated frequently. The typical effect of the lactogenic hormones on urinary Na (Fig. 5) was biphasic. A transient increase in the rate of Na excretion was curtailed by an abrupt fall in the concentration of urinary Na (Figs. 5 and 7). Thereafter, both the urine flow and the rate of excretion of Na decreased in parallel to reach minimum values in 20–35 min. Recovery in the rates of urine flow and Na excretion was even more gradual and was frequently (Fig. 7) but not always (Fig. 6) preceded by return of

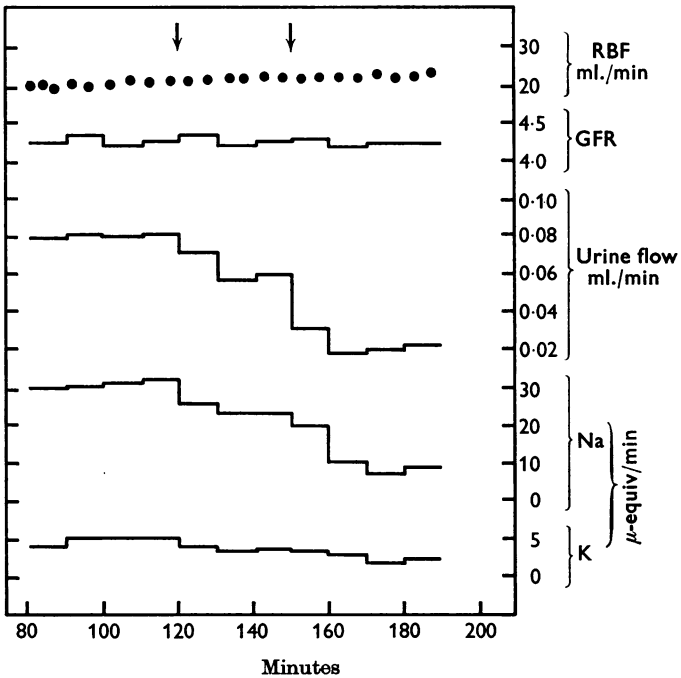

Fig. 3. Data from a kidney, 8·3 g, perfused at 134 mm Hg and 36·5° C with blood from headless donors. Ordinates and abscissae as in Fig. 1. Human growth hormone, 40 μg, and bovine growth hormone, 40 μg, were added to 150 ml. circulating blood at the first and second arrows respectively.

RBF to control levels. The early transient diuretic phase of the action of the lactogenic hormones was absent in one experiment (Fig. 6). In this experiment the effect of the lactogenic hormones on renal excretion of salt and water very closely resembled the typical effect of the growth hormones on these parameters. In each of two experiments which were characterized by urinary Na/K values of less than 3·6, the lactogenic hormones (Fig. 5) increased the urinary excretion of K. In all other experiments the lactogenic hormones, like the growth hormones, either decreased or failed to

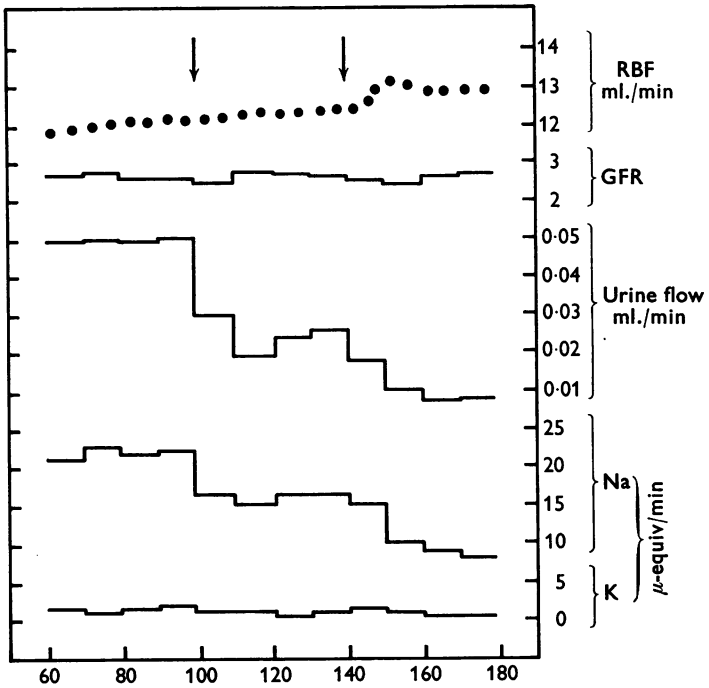

Fig. 4. Data from a kidney, 5·2 g, perfused at 126 mm Hg and 36° C with blood from headless cats. Ordinates and abscissae as in Fig. 1. Human growth hormone, 50 μg, and 2·5 μg aldosterone were added to 150 ml. blood at the first and second arrows, respectively.

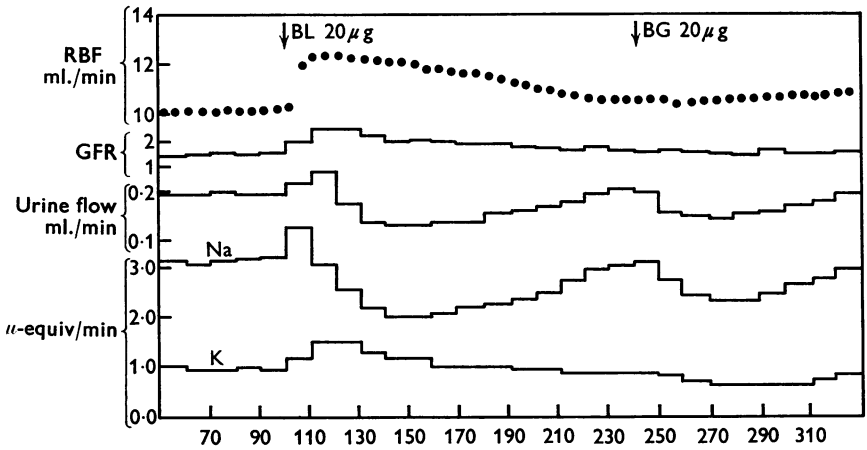

Fig. 5. Data from a kidney, 5·3 g, perfused at 115 mm Hg and 37·5° C with blood from intact donors. Ordinates and abscissae as in Fig. 1. At the first and second arrows, 20 μg of bovine lactogenic (BL) and 20 μg bovine growth (BG) hormone were added, respectively, to 150 ml. of circulating blood.

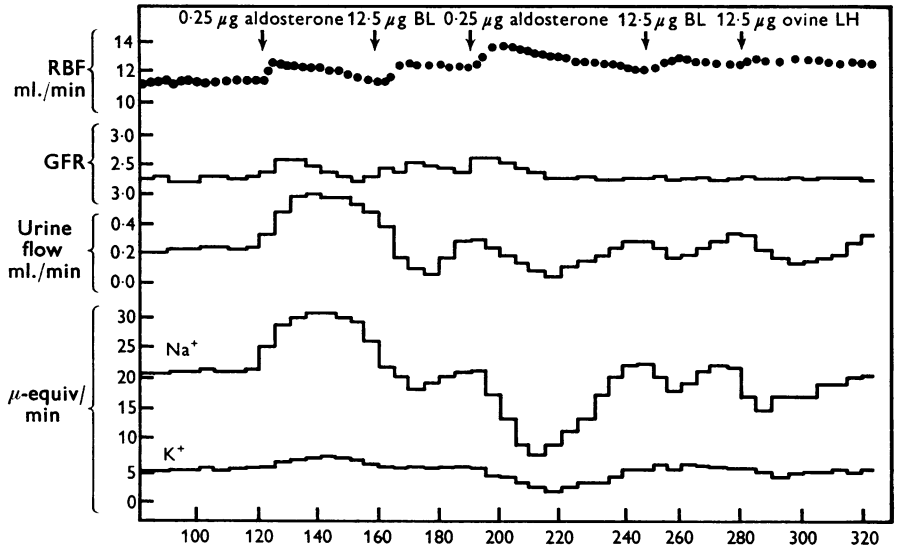

Fig. 6. Data from a kidney, 4·8 g, perfused at 112 mm Hg and 37° C with blood from headless donors. At the arrows, the following were added to 150 ml. of circulating blood: 0·25 μg aldosterone, 12·5 μg bovine lactogenic hormone, 0·25 μg aldosterone, 12·5 μg bovine lactogenic and then 12·5 μg ovine lactogenic hormone, respectively. Ordinates and abscissae as in Fig. 1.

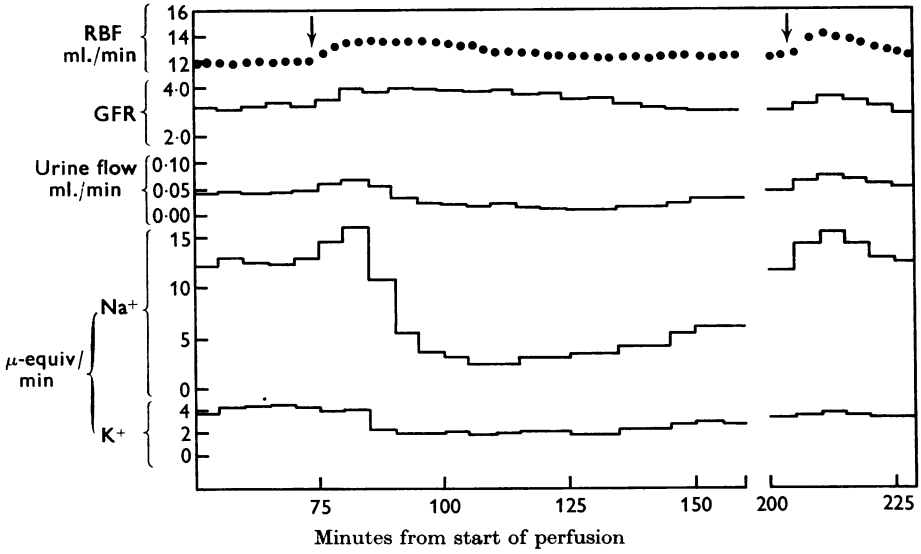

Fig. 7. Data from a kidney, 6·1 g, perfused at 124 mm Hg with blood from a headless donor. Ordinates and abscissae as in Fig. 1. 30 μg ovine lactogenic hormone and 4 mu syntocinon were added to 150 ml. circulating blood at the first and second arrows, respectively.

influence renal K excretion. The ovine lactogenic hormone was rather more effective than the bovine lactogenic hormone in production of antidiuresis and Na retention by these preparations (Fig. 6).

Lactogenic hormone, like growth hormone (Lockett & Roberts, 1963) and oxytocin (Davey & Lockett, 1960), converted the diuretic natriuretic effect of aldosterone in blood from headless animals into one of antidiuresis accompanied by retention of both Na and K (Fig. 6).

The transient early natriuresis and diuresis which accompanied the rise in GFR and RBF caused by the lactogenic hormones bore at least superficial resemblance to the action of oxytocin on these kidneys (Fig. 7).

DISCUSSION

The qualitatively similar actions of bovine, ovine and human growth hormone on the perfused kidney indicates structural similarities in these large molecules on which their renal actions are based. The relation between the intensity of their actions (ovine > bovine > human) on this preparation appears more closely related to their molecular weights which, according to Li (1960), are 48,000; 45,000 and 25,000, respectively, or possibly to the weights of the molecules corresponding to a single —S—S— bridge (9420; 11,250; 13,500, respectively) than to either N- or C-terminal sequences. (Wilhelmi, 1960; Li, 1960; Liu, Dixon & Li, 1964). The similarity between the antidiuretic sodium retaining phase of action of the lactogenic hormones and the effect of growth hormones on this preparation indicates that an identical or similar amino acid sequence, responsible for Na retention, is to be sought in both growth and lactogenic hormones. Failure to detect any effect of the vascular action of the lactogenic hormone in experiments on intact rats (Lockett & Nail, 1965) should probably be attributed to the refractoriness of the renal vascular bed which develops on repeated exposure to lactogenic hormone (Fig. 6) and/or to the large error of estimates of creatinine clearance in this species.

The ability of the growth (Lockett & Roberts, 1963) and lactogenic hormones to sensitize the nephrons to the sodium retaining action of aldosterone is shared by oxytocin (Davey & Lockett, 1960). The vascular action and transient diuretic natriuretic effects of the lactogenic hormones on the isolated kidney resemble the action of oxytocin on this preparation (Fig. 7). Moreover, non-dialysable fractions with uterine oxytocic activity can be separated from alkali treated growth and lactogenic hormones by starch-gel electrophoresis (Lockett, in preparation). It therefore seems probable that the growth and lactogenic hormones contain oxytocin-like amino acid sequences.

SUMMARY

1. The direct renal actions of human, ovine and bovine growth hormones and of ovine and bovine lactogenic hormones have been compared on cat kidneys perfused with blood from headless donors.

2. All three growth hormones reduced the rates of the urinary excretion of sodium (Na), potassium (K) and water and were without influence on renal blood flow (RBF) and the rate of glomerular filtration (GFR). The effect of 20 μg ovine was equal to that of 30 μg bovine growth hormone, and the action decay curves were similar. The maximum intensity of action of 50 μg human growth approximated to that of 30 μg bovine growth hormone. The human hormone had the shorter duration of action.

3. The lactogenic hormones increased RBF and GFR and had biphasic action on urine flow and electrolyte excretion. An initial transient diuresis accompanied by natriuresis preceded a long-lasting antidiuresis accompanied by retention of Na and K, only when the Na/K of the urine was less than 3·6, did the lactogenic hormones increase K excretion. The ovine hormone was slightly the more potent.

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REFERENCES

DAVEY, M. J. & LOCKETT, M. F. (1960). Actions and interactions of aldosterone monacetate and neurohypophysial hormones on the isolated cat kidney. *J. Physiol.* **152**, 206–219.

FERGUSON, K. A. (1964). Starch gel electrophoresis. Application to the classification of pituitary proteins and peptides. *Metabolism*, **13**, 985–1002.

FERGUSON, K. A. & WALLACE, A. L. C. (1961). Starch gel electrophoresis of anterior pituitary hormones. *Nature, Lond.*, **190**, 629–630.

LI, C. H. (1960). Studies on human pituitary growth and gonadotrophic hormones. *Ciba Foundation Colloquium on Endocrinology*, ed. G. E. W. WOHLSTENHOLME & C. M. O'CONNOR, vol. 13, pp. 46–67. London: Churchill.

LIU, W. K., DIXON, J. S. & LI, C. H. (1964). Human growth hormone X. Isolation of the aminoacid sequence of the NH_2 terminal octapeptide. *Biochim. Biophys. Acta*, **93**, 428–429.

LOCKETT, M. F. & NAIL, B. (1965). A comparative study of the renal actions of growth and lactogenic hormones in rats. *J. Physiol.* **180**, 147–156.

LOCKETT, M. F. & ROBERTS, C. N. (1963). Some actions of growth hormone on the perfused cat kidney. *J. Physiol.* **169**, 879–888.

WILHELMI, A. E. (1960). Systematic fractionation of human pituitaries. *Ciba Foundation Colloquium on Endocrinology*, ed. G. E. W. WOHLSTENHOLME & C. M. O'CONNOR, vol. 13, pp. 25–45. London: Churchill.