THE EFFECTS OF CORTICOTROPHIN, GLUCOSE AND POTASSIUM CHLORIDE ON SECRETION BY THE NASAL SALT GLAND OF THE DUCK, ANAS PLATYRHYNCHOS

M. PEAKER,* STEPHANIE J. PEAKER,† J. G. PHILLIPS‡ AND A. WRIGHT§
Department of Zoology, The University of Hong Kong

(Received 30 September 1970)

SUMMARY

Ducks given corticotrophin (ACTH) i.m. for 5 days secreted significantly more nasal fluid in response to an i.v. injection of 0.5 M-NaCl. However, blood glucose and plasma potassium concentrations also increased in the birds given ACTH and when these changes in blood composition were produced by injecting glucose or KCl, an effect similar to that of ACTH was obtained, suggesting that glucoorticoids influence the salt gland indirectly rather than, or as well as, directly. The concentrations of Na⁺ and K⁺ in the nasal fluid were decreased by ACTH, an effect not mimicked by glucose or KCl, and this might suggest some direct influence on water movements in the salt gland. ACTH increased nasal secretion in response to a minimal stimulatory salt load approximately 15 min after i.v. injection and this increase coincided with a marked rise in blood glucose concentration.

INTRODUCTION

Excretion of hypertonic sodium chloride solutions by the nasal salt glands of certain birds is of survival value in permitting the exploitation of marine and estuarine habitats (see review by Schmidt-Nielsen, 1960). Apart from parasympathetic nervous control (Fänge, Schmidt-Nielsen & Robinson, 1958; Ash, Pearce & Silver, 1966, 1969), it has been demonstrated that the concentration of circulating corticosteroids and adrenocorticotropic hormone can influence the rate of nasal secretion by the salt glands of the domestic duck. Holmes, Phillips & Butler (1961), showed that the normal extrarenal response to oral loading with hypertonic NaCl was enhanced by treatment with cortisol, deoxycorticosterone, corticotrophin (ACTH) and aldosterone. In later experiments, Phillips & Bellamy (1962) gave ducks an intra-

Present addresses:
† 24 The Close, Babraham, Cambridge.
‡ Department of Zoology, University of Hull.
§ Department of Zoology, La Trobe University, Victoria, Australia.
venous load of hypertonic saline which failed to induce urine production and which thus allowed extrarenal salt excretion to be studied separately. In birds so treated, aldosterone had no effect and it was concluded that in the earlier experiments of Holmes et al. (1961), renal retention of Na⁺ could explain the increased extrarenal response to salt-loading; cortisol, deoxycorticosterone and ACTH were still effective as was the naturally occurring hormone corticosterone (Holmes, Phillips & Chester Jones, 1963). The conclusion was therefore reached that the glucocorticoid rather than the mineralocorticoid component of the adrenal secretion was involved in the enhancement of nasal salt secretion.

It is possible that the glucocorticoids exert their effect indirectly by changing the composition of the blood rather than by affecting the salt glands directly. Therefore, the effects of exogenous ACTH on nasal secretion and on blood composition were investigated. An attempt was also made to mimic some of the effects on the composition of the blood by other means in order to determine whether these changes could account for the enhanced nasal secretion observed after treatment with ACTH.

MATERIALS AND METHODS

*Animals.* Male domestic ducks of the White Pekin variety, not less than 12 weeks old and weighing approximately 2 kg, were housed individually for several weeks before the experiments were performed. Tap water and commercial duck pellets were freely available.

*Effects of corticotrophin on nasal secretion and blood composition.* A group of five birds was treated with porcine ACTH (Acthar Gel, gelatine injections, Armour Pharmaceutical Co.). Each bird received 20 i.u. intramuscularly for 5 days and a similar amount 1 h before salt loading. This regimen was similar to that employed by Holmes et al. (1961). A control group of five birds received a gelatine solution in 0·154 M-NaCl. On the day of the experiment, a 5 ml blood sample was taken from the right metatarsal vein and 0·5 M-NaCl (18 ml/kg, Phillips & Bellamy, 1962) was injected through the same needle. The ducks were then placed in special racks and nasal fluid was collected over 15 min periods in tared cotton wool swabs for 3 h as described by Wright, Phillips & Huang (1966). After the experiment, the birds were killed and the adrenal glands removed.

Four untreated ducks were given a minimal stimulatory load of 1·7 M-NaCl as described by Peaker, Phillips & Wright (1970). After checking the validity of the control time-course of secretion the procedure was again repeated, but when the first drop of nasal fluid appeared and the infusion was stopped, 25 i.u. ACTH (Organon) in 0·2 ml 0·154 M-NaCl was injected i.v.

*Effects of glucose and KCl on nasal secretion.* A catheter was inserted into a metatarsal vein of five ducks by the method described by Peaker et al. (1970) and heparin (500 i.u./kg) was injected. The birds were then given 0·5 M-NaCl containing 0·12 M-glucose (18 ml/kg) i.v.; 2 ml blood samples were then taken at intervals of 15 or 30 min and nasal fluid was collected for 3 h. Another group was treated in a similar manner except that KCl (0·06 M) was added to the 0·5 M-NaCl used for salt loading.

*Analytical procedures.* The analysis of blood and nasal fluid has been described previously (Peaker et al. 1970; Wright et al. 1966). No urine was produced in these experiments.
**ACTH and salt-gland secretion**

**RESULTS**

**Effects of corticotrophin**

In ducks given ACTH for 5 days, nasal secretion in response to salt loading was significantly increased in terms of the amount of nasal fluid and the amount of Na excreted over a 3-h period. K⁺ excretion was not significantly altered (Table 1). The concentrations of Na⁺ and K⁺ in the secretion were significantly decreased by ACTH administration (Table 1). The time-course of secretion (Fig. 1) shows that the increased total output in the ACTH-treated birds can be attributed to the maintenance of secretion at a high rate when output from the controls was falling or had ceased.

| Table 1. Effects of corticotrophin (ACTH) (20 i.u./day for 5 days) on nasal salt-gland function in response to 0·5 m-NaCl, blood composition before salt loading and adrenal weight (means ± S.E.M.; 5 birds in each group) |
|---------------------------------|---------------------------------|-------|
| Nasal secretion in 3 h          | Sham-treated                   | ACTH-treated                   | P     |
| Weight (g)                     | 11·0±1·1                       | 19·7±0·9                       | <0·001|
| Na⁺ (mequiv.)                  | 4·9±0·6                        | 7·5±0·8                        | <0·05 |
| K⁺ (mequiv.)                   | 0·15±0·03                      | 0·22±0·05                      | NS    |
| Nasal fluid concentration      |                                 |                                 |       |
| Na⁺ (mequiv./l)                | 445·0±10*                      | 380·0±10                       | <0·01 |
| K⁺ (mequiv./l)                 | 13·9±0·6*                      | 11·4±0·5                       | <0·01 |
| Haematocrit (%)                | 43·8±1·0                       | 39·6±1·2                       | <0·05 |
| Blood glucose (mg/100 ml)      | 105·0±3·8                      | 132·0±6·0                      | <0·01 |
| Plasma K⁺ (mequiv./l)          | 3·40±0·06                      | 3·75±0·10                      | <0·05 |
| Plasma Na⁺ (mequiv./l)         | 148·3±2·1                      | 147·2±2·2                      | NS    |
| Adrenal weight (mg)            | 126·1±8·2                      | 184·5±3·7                      | <0·001|
| (mg/kg) body weight            | 60·2±2·6                       | 73·9±2·5                       | <0·01 |

* Figures were combined with those of another group (Table 2) since there was no significant difference between the values obtained in this experiment.

NS = not significant.

ACTH significantly increased plasma K⁺, blood glucose and adrenal weight and significantly decreased the haematocrit but had no effect on plasma Na⁺ (Table 1).

ACTH injected i.v. enhanced nasal secretion in all the four birds which had received a minimal stimulatory load of salt. The increase in secretion above the normal values started 10–20 (mean 15 min) after ACTH had been given (Fig. 2) and coincided with a marked increase in the blood glucose concentration which persisted for at least 1 h. Secretion was maintained at a high level for 55–75 min and thereafter fell to cease 85–100 min after secretion started. Normally, the birds stopped secreting 35–45 min after the first drop of fluid had appeared. It might be argued that shortly after the onset of secretion, the nasal gland was producing fluid at its maximum rate and that more ACTH would fail to have an effect. However, prolactin (Peaker et al. 1970) increased secretion within the first 5 min so that this argument would appear to be invalidated.

**Effects of glucose**

When birds were given glucose i.v. with the salt load, the blood glucose concentration increased (Fig. 3). Nasal secretion at a high rate was prolonged (Fig. 1) and therefore the amount of fluid collected over 3 h increased significantly from 11·0±1·1
Fig. 1. Mean effects of corticotrophin (ACTH) (○), glucose (●) and KCl (▲) on nasal secretion in ducks given 0·5 m-NaCl i.v. at 0 h (arrow). ACTH was given for 5 days (20 i.u./day). Glucose (0·12 m) or KCl (0·06 m) was dissolved in the 0·5 m-NaCl injected to induce secretion. Control birds (□) were given 0·5 m-NaCl only.

Fig. 2. Effects of 25 i.u. corticotrophin (ACTH) (given at 0 min, arrow) on nasal secretion in a duck given a minimal stimulatory salt load in the manner described by Peaker et al. (1970). 10 % NaCl was infused at 1 ml/min until nasal secretion started at time 0 min.
(s.e.m.) g to 18.6 ± 1.0 g (P < 0.01). Similarly, the output of Na⁺ but not K⁺ was significantly increased (Table 2). The concentrations of Na⁺ and K⁺ in the nasal fluid were not significantly altered (Table 2).

Table 2. Effects of i.v. glucose and KCl on nasal salt-gland secretion in response to an i.v. salt load (means ± s.e.m.; 5 birds in each group)

<table>
<thead>
<tr>
<th>Salt load</th>
<th>Concentration (mequiv./l)</th>
<th>Concentration (mequiv./l)</th>
<th>Concentration (mequiv./l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight (g)</td>
<td>Na⁺ (mequiv.)</td>
<td>K⁺ (mequiv.)</td>
</tr>
<tr>
<td>0.5 M-NaCl</td>
<td>11.0 ± 1.1</td>
<td>4.9 ± 0.5</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td>0.5 M-NaCl in 0.12 M-glucose</td>
<td>18.6 ± 1.0**</td>
<td>9.1 ± 0.7**</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td>0.5 M-NaCl in 0.06 M-KCl</td>
<td>24.2 ± 1.2***</td>
<td>10.9 ± 1.0***</td>
<td>0.37 ± 0.04**</td>
</tr>
</tbody>
</table>

P values with respect to the group given only NaCl: **P < 0.01; ***P < 0.001; no other differences were significant.
† 8 observations.

Fig. 3. Effects of 0.5 M-NaCl (18 ml/kg) and 0.5 M-NaCl containing 0.12 M-glucose injected i.v. at zero time on blood glucose concentrations in ducks. Vertical bars represent ± s.e.m.

**Effects of KCl**

The administration of KCl had similar, but more marked, effects in enhancing secretion in response to glucose (Table 2; Fig. 1). Plasma samples were taken only after 3 h and the [K] was found to be significantly higher (P < 0.02) in the birds given KCl (4.4 ± 0.1 mequiv./l) than in the controls (3.9 ± 0.11 mequiv./l).

**DISCUSSION**

The results confirm the findings of Holmes et al. (1961) and Phillips & Bellamy (1962) that ACTH and therefore glucocorticoid hormones influence nasal salt secretion in the duck. However, the composition of the blood was also changed and such changes, if induced by means other than ACTH, themselves enhanced secretion and thus mimicked the action of ACTH and glucocorticoids. Therefore, it need not be postulated that the glucocorticoids are exclusively exerting their effect directly on the cells of the salt gland as has been supposed (Holmes et al. 1963). While the effect of ACTH in enhancing nasal secretion could be attributed to an indirect action, the alternative possibility that the glucocorticoids may also
have a direct influence on the salt gland cannot be ruled out. The concentration of
the nasal fluid was decreased by ACTH and glucocorticoids in these as well as in
the experiments of Holmes et al. (1961) but the administration of glucose or KCl had
no significant effect on the concentration. Therefore, the possibility of a direct effect
on the permeability of the secretory tubules to water must be considered, but
whether corticosteroids normally play some part in affecting concentration or whether
the effect is induced only by greatly raised circulating hormone levels is not known.

There is the further possibility that corticosteroids act in a direct but different
manner to that already proposed. This possibility gains support from the fact that
Macchi, Phillips & Brown (1967) showed that after the administration of hypertonic
saline i.v. the corticosterone levels in systemic blood remain unchanged but that the
pituitary–adrenal system is activated in that the maintenance of an unchanged level
of corticosterone in the plasma is achieved despite a markedly expanded plasma
volume. The functional significance of these endocrine changes may be twofold
(Phillips, 1968). The maintenance of corticosterone at near normal levels under
conditions of excess salt intake might be a requirement for the physiological processes
known to be affected by corticosteroids but which are unconnected with the animal’s
immediate problem of homeostasis in terms of electrolyte and water balance. On the
other hand, this may also be important in providing the nasal gland with sufficient
corticosterone for the trapping process of the nasal gland (Phillips & Bellamy, 1966;
Bellamy & Phillips, 1966) which results in the accumulation of corticosterone by
the cells of the salt gland. This local increase in the corticosterone concentration in
the nasal gland may have a functional significance and may be achieved in part by the
great increase in blood flow through the gland which occurs when the tissue is active
(Phillips & Bellamy, 1966). The final role of corticosteroids in the nasal gland might
therefore be in the stimulation of DNA- and RNA-dependent protein synthesis, an
aspect of the cellular activity of corticosteroids which has been reviewed by Holmes,

The mechanism by which glucose and KCl enhance nasal secretion is not clear. It
seems unlikely that extra glucose supplies more energy to the gland. The blood flow is
very high (about 10 ml/g/min, Hanwell, Linzell & Peaker, 1970) and from the known
requirements of sodium pumps, the amount of glucose that would probably be
normally extracted by the secreting gland may only be a very small percentage of the
amount arriving in the arterial blood. Thus the supply of glucose to the secretory
mechanism is not likely to be rate-limiting. Glucose may act by raising the tonicity of
the blood and thereby affect the proposed osmoreceptors (Ash, 1969). An additional
mechanism may account for the effect of K+. There is some evidence that secretion
can start at lower plasma levels of Na when the plasma K concentration is raised
(Ash, 1969; A. Hanwell, J. L. Linzell & M. Peaker, unpublished observations) and it
seems possible that the receptors or ganglia in the efferent nervous pathway are
affected by small increases in external K+. In this respect it is interesting to note that
Holmes et al. (1961) found that the onset of secretion occurred at lower plasma Na+
concentrations after treatment with cortisol and ACTH and that both these steroids
markedly raised the plasma K+ concentration before salt loading.
During the course of this work M. P. was an S.R.C. NATO Scholar. The work formed part of a research programme supported by grants to J. G. P. from the Nuffield Foundation and Sir Shiu Kin Tang. We are grateful to Mr H. Kadoorie, O.B.E., Chev. Leg. Hon. of the Kadoorie Agricultural Aid Association of Hong Kong, for the generous gift of the ducks used in these experiments and to Mr H. C. Leung and Mr F. S. Lam for their diligent care of the experimental animals.

REFERENCES