GENERAL AND COMPARATIVE ENDOCRINOLOGY 48, 310–318 (1982)

Ontogeny of Endocrine Control of Osmoregulation in Chick Embryo

II. Actions of Prolactin, Arginine Vasopressin, and Aldosterone

BYRON A. DONEEN AND THOMAS E. SMITH

Division of Biological Sciences, University of Michigan, Ann Arbor, Michigan 48109

Accepted October 16, 1981

Actions of ovine prolactin (oPRL), arginine vasopressin (AVP), and aldosterone on volume of allantoic fluid and its Cl^- concentration and on renal Na^+-K^+-ATPase activities in mesonephros and metanephros were examined at various times during ontogeny of the chick embryo. Ovine PRL decreased allantoic fluid volume and lowered Cl^- concentration in this compartment when injected for various periods prior to 14 days of incubation. After this time, when endogenous PRL levels become elevated (Day 16), exogenous oPRL no longer altered allantoic fluid volume or Cl^-. Ovine PRL also stimulated Na^+-K^+-ATPase activity in the metanephros when embryos were treated before 14 days of incubation but did not stimulate the transport enzyme in mesonephros. Stimulation of metanephric Na^+-K^+-ATPase was a direct action of oPRL since this activity was also stimulated by the hormone in metanephros maintained in organ culture for 3 days. PRL did not stimulate Na^+-K^+-ATPase in organ-cultured mesonephros, and it failed to stimulate Na^+-K^+-ATPase in metanephros of juvenile chickens treated after hatching. Injection of AVP, a known contaminant of the NIH–oPRL used in these experiments, duplicated some, but not all actions of oPRL in the embryo. Like oPRL, AVP reduced the Cl^- concentration of allantoic fluid, but AVP did not mimic the effect of oPRL in stimulation of metanephric Na^+-K^+-ATPase activity. Thus, oPRL preparations appear to possess osmoregulatory actions in the embryo which can be distinguished from actions attributable to the contaminant, ADH. Aldosterone injected prior to 16.5 days of incubation depressed activities of Na^+-K^+-ATPase and Mg^{2+}-ATPase in mesonephros, but did not alter these enzyme activities in metanephros. The depressed enzyme activities induced by aldosterone in mesonephros may have been, in part, a toxic response to the hormone as control injections of cholesterol had comparable effects. On the other hand, aldosterone specifically stimulated Ca^{2+}-dependent ATPase(s) and succinate dehydrogenase in metanephros. The three principal osmoregulatory hormones of adult birds, PRL, ADH, and aldosterone, seem to be active also during ontogeny of the avian embryo, with mesonephric and metanephric kidneys as target organs.

In the first paper in this series (Doneen and Smith, 1982), it was shown that the pituitary gland was required for normal distribution of fluid and ions in the chick embryo and also influenced Na^+-K^+-ATPase activity in embryonic kidneys. However, it was also shown that some osmoregulatory defects could be indirect consequences of the severely disturbed metabolism in the hypophysectomized embryo. In this paper, some limitations of the hypophysectomized model are circumvented by using two alternative experimental strategies to study developmental roles of pituitary hormones having known osmoregulatory functions in birds, prolactin (PRL) and ADH (Ensor, 1975; Peaker, 1979). The major emphasis of this report is placed on PRL since this hormone has important developmental roles in amphibians (Bern, 1975), and has been implicated as having an osmoregulatory function during ontogeny in other higher vertebrates, including the fetal mammal (reviewed by Clarke and Bern, 1980). The first strategy was to treat intact embryos with ovine prolactin (oPRL) prior to the time in development when endogenous hormone secretion commences, and in this way to

0016-6480/82/110310-09$01.00/0

attempt a precocious acceleration of normal endocrine responses. The second experimental strategy sought to detect direct actions of oPRL by treating two potential embryonic target organs, mesonephric and metanephric kidneys, in organ culture. One experiment was designed to detect the osmoregulatory actions of AVP, a known contaminant of the oPRL (NIH PS-12) used in this work (Malarkey *et al.*, 1975; North *et al.*, 1979). The actions of the third major avian osmoregulatory hormone, aldosterone (Holmes and Pearce, 1979), was also investigated in the embryo. These experiments were focused on endocrine regulation of kidney function in the embryo. Thus, in most experiments, measurements have been limited to volume of allantoic fluid (elaborated by kidneys, in part) and its ionic composition, and to certain enzymes of the kidney known or believed to be important in monovalent and divalent ion transport or in the synthesis of ATP required to power transport enzymes. Enzymes analyzed included Na^+-K^+-ATPase, Ca^{2+}-ATPase, and mitochondrial succinate dehydrogenase whose importance in renal function has been recently reviewed by Perez-Gonzalez de la Manna *et al.* (1980).

MATERIALS AND METHODS

Hormone treatment of embryos and chicks. Fertilized eggs were windowed at 36–40 hr of incubation as described in the preceding paper (Doneen and Smith, 1982). Ovine prolactin (NIH PS-12) and AVP (Sigma) were dissolved in 0.9% sterile saline, pH 7.5. Aldosterone hemisuccinate and cholesterol (Sigma) were dissolved in a small volume of ethanol (ETOH) and then diluted to the final concentration with sterile 0.9% saline (final ETOH 2%). Control treatments consisted of 0.9% saline also containing 2% ETOH, in the aldosterone experiment. Hormones were applied to a well-vascularized area of the chorioallantoic membrane of windowed eggs in volumes of 50 μl. Windowed eggs contained a small taped hole in the shell which could be uncovered to expose the chorioallantoic membrane. The treatment schedules are presented under Results. Tissue and allantoic fluid samples were collected, stored (tissues at −70°, fluid samples at −20°), and analyzed as previously described (Doneen and Smith, 1982). In one experiment, hatched chicks were injected daily intramuscularly with two different doses of oPRL (150 μg/day ≅ 1.5 μg/g body wt or 750 μg/day ≅ 7.5 μg/g body wt). Control injections were 750 μg/day of BSA (bovine serum albumin) which equaled about 7.5 μg/g body wt based on the weight of the animal on the first day of injection. Injections began on Day 11 following hatching and continued through Day 20 of life. Birds were sacrificed by a blow to the head on Day 21, kidneys collected and frozen for subsequent enzyme assay.

Organ culture. Mesonephric and metanephric kidneys were dissected separately from embryos at 13.5 days of incubation. Tissues were minced into small pieces (~ 1 to 2 mm^3) and transferred to medium 199 with Earles' Salts, pH 7.5 (GIBCO), and maintained at 39° for 3 days in an atmosphere of 95% Co_2–5% CO_2. Both types of kidneys were divided into three treatment groups in which culture medium was variously supplemented: (1) No further additions; (2) insulin (5 μg/ml) + corticosterone acetate (1 μg/ml); (3) Insulin + corticosterone acetate + oPRL (2.5 μg/ml).

Enzyme assays. Renal Na^+-K^+-ATPase activity was assayed in fresh and cultured tissues as described previously (Doneen and Smith, 1982). Mg^{2+}-ATPase is the ouabain-insensitive component measured in the same assay. Kidney Ca^{2+}-ATPase was measured according to Fenwick (1979). In brief, 27–75 μg of protein was added to 1.85 ml of 20 m*M* Tris–HCl, 70 m*M* NaCl, 4 m*M* $CaCl_2$, pH 7.4. The reaction was started by addition (to 5 m*M*) of vanadate-free Na_2ATP. Reactions were stopped with 2 ml cold 10% trichloroacetic acid. In both ATPase assays, enzymatically liberated phosphate (P_i) was measured essentially as described by Peterson (1978). Succinate dehydrogenase was measured using iodotetranitrazolium as electron acceptor and monitoring its conversion spectrophotometrically to the formazan product as described by Clark and Porteus (1964). Enzyme reactions were conducted at 39°. Protein concentration was determined using the coomassie blue reaction (Bio-Rad) with bovine γ-globulin as standard.

Statistics. Significance of differences between means of control and experimental groups was analyzed using Student's *t* test (two-sided). In cases of unequal variances, the adjusted *t* test was employed (Battacharyya and Johnson, 1977).

RESULTS

The first three experiments were concerned with actions of oPRL when injected at various times during incubation and after hatching. In Table 1, effects of oPRL (20 μg/day) in the 9.5-day-old embryo (injections on Days 6, 7, 8) and in the 16.5-day-old embryo (injections on Days 11, 13, 15)

TABLE 1
EFFECTS OF oPRL TREATMENT PRIOR TO DAYS 9.5 AND 16.5 OF INCUBATION ON VOLUME AND Cl^- CONCENTRATION OF ALLANTOIC FLUID

Incubation time (days)	Treatment[a]	n	Allantoic fluid volume (ml)	Allantoic fluid Cl^- (meq/liter)
9.5	Saline	5	3.9 ±0.4	73.0 ± 3.0
	oPRL	6	2.9 ± 0.3*	55.45 ± 4.2***
16.5	Saline	5	6.5 ± 0.7	51.2 ± 6.3
	oPRL	5	5.9 ± 0.4^{NS}	47.9 ± 4.2^{NS}

Note. Values are means ± SE; n, number of embryos. NS, not significantly different from saline control.

[a] Ovine PRL treatment schedules: 20 μg/day on Days 6, 7, 8, for 9.5-day-old embryos (~8 to 12.5 μg/g wet wt of embryo); 20 μg/day on Days 11, 13, 15, for 16.5-day-old embryos (~2.0 to 5.5 μg/g wet wt of embryo); control injections, 50 μl 0.9% saline.

* $P \leq 0.05$.

*** $P \leq 0.001$.

on volume of allantoic fluid and its Cl^- concentration are compared. In the younger embryos, oPRL reduced allantoic fluid volume by 25% ($P \leq 0.05$) and sharply lowered its Cl^- concentration ($P \leq 0.001$). In the older embryos, in contrast, oPRL did not alter either aspect of allantoic fluid.

A summary of several measures of osmoregulatory function determined after separate treatments with oPRL or with

TABLE 2
EFFECTS OF oPRL AND AVP ON ALLANTOIC FLUID VOLUME, ON Cl^- CONCENTRATION IN ALLANTOIC FLUID AND SERUM, AND ON TRANSPORT ATPases OF MESONEPHROS AND METANEPHROS IN THE 14.5-DAY-OLD EMBRYO

Treatment[a]	n:	Saline 5	oPRL 5	AVP 5
Allantoic fluid volume (ml)		5.8 ± 1.5	5.4 ± 1.8 ^{NS}	7.3 ± 1.3^{NS}
Allantoic fluid Cl^- (meq/liter)		57.4 ± 4.1	41.9 ± 5.1 **	39.3 ± 5.4**
Serum Cl^- (meq/liter)		103.4 ± 4.9	98.5 ± 3.0 ^{NS}	101.1 ± 4.2^{NS}
Na^+-K^+-ATPase				
(μmol P_i mg protein^{-1} hr^{-1})				
Mesonephros		1.98 ± 0.27	3.10 ± 0.62^{NS}	2.21 ± 0.47^{NS}
Metanephros		1.39 ± 0.23	2.48 ± 0.25**	1.33 ± 0.11
Mg^{2+}-ATPase				
(μmol P_i mg protein^{-1} hr^{-1})				
Mesonephros		7.58 ± 0.89	7.89 ± 0.71^{NS}	6.90 ± 0.81^{NS}
Metanephros		3.41 ± 0.28	4.44 ± 0.54^{NS}	4.23 ± 1.18^{NS}
Ca^{2+}-ATPase				
(μmol P_i mg protein^{-1} hr^{-1})				
Mesonephros		5.62 ± 0.55	5.42 ± 0.32^{NS}	3.03 ± 0.47**
Metanephros		2.21 ± 0.03	2.30 ± 0.41^{NS}	3.15 ± 0.30**

Note. Values are means ± SE; n, number of embryos.

[a] Treatment schedules: oPRL, 20 μg/day on Days 8, 10, 12, and 13 of incubation (~2.5 to 7.5 μg/g wet wt of embryo); AVP, 0.2 ng/day on Days 8, 10, 12, and 13 of incubation (this AVP dose duplicates the radioimmunoassayable levels of AVP in the NIH oPrl (PS-12) (Malarky *et al.*, 1975; North *et al.*, 1979); controls, 50 μl 0.9% saline.

** $P \leq 0.01$; NS, not significantly different from control.

TABLE 3
EFFECTS OF oPRL TREATMENT ON Na^+-K^+-ATPASE ACTIVITY IN METANEPHROS OF 3-WEEK-OLD CHICKENS

Treatment[a]	n	Na^+-K^+-ATPase (μmol P_i mg protein^{-1} hr^{-1})
Control—BSA (750 μg/day × 10 days)	5	4.91 ± 0.66
oPRL (150 μg/day × 10 days)	5	5.16 ± 0.63
oPRL (750 μg/day × 10 days)	5	5.39 ± 0.50

Note. Values are means ± SE; n, number of embryos. No significant differences were observed.

[a] Treatment schedule: Beginning on Day 11 after hatching, chickens were treated with BSA (750 μg/day; ~7.5 μg/g body wt), or oPRL at 150 μg/day (~1.5 μg/g) or 750 μg/day (~7.5 μg/g) for 10 days. Kidney samples were collected at 21 days of age.

AVP prior to Day 14.5 of incubation is found in Table 2. The amount of AVP injected quantitatively duplicated the AVP contamination (0.01% by weight) known to exist in NIH-oPRL (NIH PS-12; Marlarkey *et al.*, 1975; North *et al.*, 1979). These results show that some actions of the contaminated oPRL, but not all, can be obtained by treatment with AVP alone. Neither treatment altered allantoic fluid volume or serum Cl^- concentration in the 14.5-day-old embryo. Both, however, significantly reduced the Cl^- concentration of allantoic fluid ($P \leq 0.01$; Table 2). Each treatment produced different patterns of activity in modulation of kidney enzymes. oPRL stimulated Na^+-K^+-ATPase in metanephros, but AVP did not. The tendency of oPRL to elevate Na^+-K^+-ATPase in mesonephros was not statistically significant (Table 2). On the other hand, AVP given alone significantly lowered Ca^{2+}-ATPase activity in mesonephros, and elevated this activity in metanephros ($P < 0.01$; Table 2). Paradoxically, oPRL, though contaminated with AVP, showed no similar enhancement or depression of kidney Ca^{2+}-ATPases. Neither treatment changed renal Mg^{2+}-ATPase from control levels (Table 2).

One experiment investigated effects of long-term oPRL treatment on kidney Na^+-K^+-ATPase activity in chicks after hatching. At this stage, the chicken kidney consists of the metanephros alone. Results in Table 3 show that two different doses of oPRL were without effect on renal Na^+-K^+-ATPase.

A second experimental strategy was used to detect possible direct actions of oPRL on Na^+-K^+-ATPase in the two embryonic kidneys. Mesonephros and metanephros of 13.5-day-old embryos were maintained for 3 days in organ-culture media supplemented with or lacking hormones. Insulin and corticosterone were added to media in the expectation that metabolic hormones might be necessary for viability of explants, but no direct evidence that such an *in vitro* requirement actually exists was sought or gained. The results presented in Table 4 indicate that activities of Na^+-K^+-ATPase in mesonephros were not different in tissues cultured without hormonal supplementation, or maintained with added insulin and corticosterone acetate, or with these hormones plus oPRL. The effects of the various treatments on Na^+-K^+-ATPase in organ-cultured metanephros were more complex. Insulin and corticosterone acetate alone depressed activity of Na^+-K^+-ATPase ($P \leq 0.01$), whereas oPRL, in the presence of the metabolic hormones, restored enzyme activity ($P \leq 0.01$) to the higher untreated level (Table 4).

The final experiment investigated actions

TABLE 4
EFFECTS OF 3 DAYS oPRL TREATMENT ON Na^+-K^+-ATPASE ACTIVITY IN ORGAN-CULTURED KIDNEYS FROM 13.5-DAY-OLD-EMBRYOS

Treatment	n	Na^+-K^+-ATPase (μmol P_i mg protein^{-1} hr^{-1}) Mesonephros	Metanephros
No added hormone	5	4.06 ± 0.64	3.22 ± 0.33
Insulin (5 μg/ml) and corticosterone acetate (1 μg/ml)	5	4.17 ± 0.33^{NS}	2.42 ± 0.19**
oPRL (2.5 μg/ml) + insulin and corticosterone acetate	5	4.13 ± 0.49^{NS}	3.17 ± 0.25^{NS,††}

Note. Values are means ± SE; n, number of replicates; NS, not significantly different from control (No added hormone);

** $P \leq 0.01$ compared with control.

†† oPRL group significantly different ($P \leq 0.01$) from insulin and corticosterone acetate group.

of aldosterone on allantoic fluid and on activities of four renal enzyme activities in the 16.5-day-old embryo. Control treatments in this experiment included both saline and cholesterol (steroid) injections. Aldosterone (10 μg/day on Days 12–15 of incubation) did not alter allantoic fluid volume, its Cl^- concentration, or the Cl^- concentration of serum in the 16.5-day-old embryo (Table 5). Administration of aldosterone hemisuccinate, however, produced several changes in activities of renal enzymes. In mesonephros (Table 6), aldosterone reduced rates of Na^+-K^+-ATPase and Mg^{2+}-ATPase activity. This may have been partly a nonspecific or toxic response to the hormone since cholesterol treatment produced similar reductions in these activities. On the other hand, neither aldosterone nor cholesterol effected Ca^{2+}-ATPase or SDH activities in mesonephros (Table 6). In the metanephros of 16.5-day-old embryos, prior treatment with aldosterone elevated Ca^{2+}-ATPase ($P \leq 0.01$) and SDH ($P \leq 0.01$); in this case, cholesterol administration failed to duplicate the aldosterone responses. Activities of Na^+-K^+-ATPase and Mg^{2+}-ATPase in metanephros were not changed from control values by aldosterone or by cholesterol (Table 6).

DISCUSSION

These results show that the major osmoregulatory hormones of adult birds, PRL, ADH, and aldosterone, also act during ontogeny in the avian embryo. Each hormone appeared to regulate some aspect of kidney function as inferred from changes

TABLE 5
EFFECTS OF ALDOSTERONE AND CHOLESTEROL TREATMENT ON ALLANTOIC FLUID VOLUME AND Cl^- CONCENTRATION AND ON SERUM Cl^- IN THE 16.5-DAY-OLD EMBRYO

Treatment[a]	n	Allantoic fluid volume (ml)	Allantoic Cl^- (meq/liter)	Serum Cl^- (meq/liter)
Saline	5	9.6 ± 0.8	47.6 ± 3.4	105.3 ± 5.1
Cholesterol	5	9.7 ± 0.5	43.5 ± 2.3	106.9 ± 3.6
Aldosterone-hemisuccinate	6	9.4 ± 1.1	43.2 ± 3.3	107.7 ± 1.6

Note. Values are means ± SE; n, number of embryos. No significant differences were observed.

[a] Treatment schedule: Cholesterol (steroid control; 10 μg/day) and aldosterone hemisuccinate (10 μg/day) were injected on Days 12–15 of incubation. Control injections were 0.9% saline (with 2% ETOH).

TABLE 6
EFFECTS OF ALDOSTERONE AND CHOLESTEROL ON RENAL ATPASES AND SUCCINATE DEHYDROGENASE IN MESONEPHROS AND METANEPHROS OF THE 16.5-DAY-OLD EMBRYO

Treatment[a]	n	Renal ATPases (μmol P_i mg protein^{-1} hr^{-1})			SDH (nmol formazan) (mg protein^{-1} hr^{-1})
		Na^+-K^+-ATPase	Mg^{2+}-ATPase	Ca^{2+}-ATPase	
Mesonephros					
Saline	5	3.85 ± 0.90	5.90 ± 1.93	9.01 ± 1.76	389.2 ± 81.9
Cholesterol	5	1.57 ± 0.24**	1.86 ± 0.34**	8.9 ± 1.13^{NS}	327.5 ± 38.8^{NS}
Aldosterone-hemisuccinate	6	1.65 ± 0.56**	2.48 ± 0.65**	8.6 ± 1.06^{NS}	459.1 ± 34.3^{NS}
Metanephros					
Saline	5	1.68 ± 0.28	2.47 ± 0.46	4.45 ± 0.72	163.1 ± 16.4
Cholesterol	5	1.76 ± 0.17^{NS}	2.56 ± 0.37^{NS}	4.42 ± 0.65^{NS}	218.5 ± 19.8^{NS}
Aldosterone-hemisuccinate	6	1.56 ± 0.31^{NS}	2.43 ± 0.30^{NS}	6.58 ± 0.77**	296.0 ± 23.5**

Note. Values are means ± SE; n, number of embryos.
[a] Treatment schedule: Injection schedule and doses as in Table 5.
** $P \leq 0.01$; NS, not significantly different from saline control.

following treatment in the volume of allantoic fluid or its Cl^- concentration, and from actions on renal enzymes, some concerned directly with tubular ion and water reabsorption. Other potential target organs exist for the three hormones, and these may also function in the elaboration or modification of allantoic fluid. But embryonic organs (such as gut and cloaca) and extraembryonic membranes were not investigated in this study.

Ovine PRL reduced allantoic fluid volume when injected for three days prior to Day 9.5 of incubation (Table 1), but failed to have a similar action on subsequent days (Day 14.5, Table 2; Day 16.5, Table 1). Treatment with PRL reduced allantoic fluid Cl^- in the 9.5-day-old embryo (Table 1), and also in 14.5-day-old embryos which had been treated four times beginning on Day 8 of incubation (Table 2). However, oPRL failed to lower Cl^- concentration in the 16.5-day-old embryo, in which treatment commenced on Day 11 (Table 1). In the 14.5-day-old embryo, the capacity of NIH-oPRL (PS-12) to lower Cl^- concentration of allantoic fluid could be duplicated by AVP administered alone in quantities which equalled its contamination in oPRL (0.01% by weight). The need to consider osmoregulatory effects of the small, but potent AVP contamination in some oPRL preparations has been urged before (Clarke and Bern, 1980), and results in the chick embryo confirm the sagacity of that advice. It cannot be decided whether reduced allantoic fluid volume in the 9.5-day-old embryo (Table 1) was a response to oPRL or to its contaminant, since treatment with ADH alone in older embryos caused the opposite response, a slight (statistically insignificant) increase in allantoic fluid volume (Table 2). Thus, oPRL might have produced reduced fluid volume in the younger embryos. These results do not eliminate the possibility that both PRL and ADH might act independently to lower allantoic fluid Cl^-. Resolution of possible interactions of this sort will require use of an AVP-free PRL preparation (North *et al.*, 1979).

Ovine PRL did have one osmoregulatory effect which was not duplicated by AVP. This was the stimulation of Na^+-K^+-ATPase in the metanephros of the chick embryo (Table 2). PRL in the presence of insulin and corticosterone also stimulated Na^+-

K^+-ATPase in organ-culture metanephros when compared with control kidneys which had been exposed to insulin and corticosterone acetate alone (Table 4). However, oPRL elevated metanephric Na^+-K^+-ATPase activity in culture to a level nearly identical with the rate in tissues not exposed to any hormones. Therefore, oPRL seemed to antagonize the reduction in enzyme activity elicited by insulin, by corticosterone, or by both metabolic hormones. The actions of the metabolic hormones may have been physiological or pharmacological in nature, but their actions were specific to metanephros, no similar reduction in Na^+-K^+-ATPase having been observed in mesonephros (Table 4). Most evidence supports the contention that oPRL acts directly on the metanephros in stimulation of Na^+-K^+-ATPase in the embryo. On the other hand, after hatching, injection of oPRL did not stimulate Na^+-K^+-ATPase in the metanephros of juvenile chickens (Table 3). Because circulating titers of endogenous oPRL are elevated after hatching (Harvey *et al.*, 1979), further stimulation by exogenous hormone may not have been possible. oPRL appears to be saluretic and antidiuretic in some species of adult birds (Ensor, 1975). Since stimulation of Na^+-K^+-ATPase in the metanephric kidney might be associated with increased salt as well as water retention by the embryo, some actions of oPRL in the embryo may differ from those observed after hatching. However, the amount of data available on the renal actions of PRL at any life stage are sufficiently scant to induce caution in drawing conclusions. The ability of oPRL to stimulate Na^+-K^+-ATPase in metanephros might be related to the high levels of specific PRL-receptors in kidneys of adult birds detected by White and Nicoll (1980). Prolactin did not act on mesonephric Na^+-K^+-ATPase or on the Mg^{2+}-dependent and Ca^{2+}-dependent ATPases of this embryonic kidney (Table 2 and 4). However, these activities were the only specific aspects of mesonephric function observed and the hormone may have other actions in this kidney, perhaps on water permeability or on kidney metabolism. Since actions of PRL have been identified in the mesonephric kidneys of freshwater-adapted fishes (Clarke and Bern, 1980) and some amphibians (White and Nicoll, 1979), further study of a wider range of physiological properties in the transitional mesonephros of the chick embryo seems advisable.

AVP has potent osmoregulatory actions in the chick embryo even when administered in doses as small as 0.2 ng/day. All or some of the ability of oPRL preparations to reduce allantoic fluid Cl^- concentration could be attributed to small contaminating amounts of AVP contained in the preparation used. But AVP had additional renal actions not shared with oPRL, especially on Ca^{2+}-ATPase which has been implicated in renal Ca^{2+} transport (Kinne-Saffran and Kinne, 1974; Perez de la Manna *et al.*, 1980). AVP depressed Ca^{2+}-ATPase in mesonephros, but stimulated this activity in metanephros (Table 2). This action of AVP may have been an indirect or pharmacological one, since ADH is generally believed to act on kidney distal tubules and other transporting epithelia of mammals and amphibians by modification of permeability to water and Na^+, though the molecular mechanisms involved seem to require Ca^{2+} (Hardy, 1978). In birds, ADH (arginine vasotocin) may also regulate glomerular filtration rate as well as distal water reabsorption (Holmes and Pearce, 1979; Peaker, 1979). It is of some interest that AVP, when injected with oPRL (as necessarily occurs with contaminated preparations), failed to alter renal Ca^{2+}-ATPase activities (Table 2). This suggests that PRL might also exert control on Ca^{2+}-dependent ATPases, perhaps to oppose AVP actions. Some evidence has implicated PRL as favoring renal absorption of Ca^{2+} in freshwater fishes (Pang *et al.*, 1978; Wendelaar Bonga and van der Meij, 1980), and in Ca^{2+}

homeostasis in mammals (Mainoya, 1975; Pahuja and deLuca, 1981), but it cannot yet be stated that PRL has a similar role in the avian embryo.

Like PRL and AVP, aldosterone displayed actions which differed in the two types of kidneys of the chick embryo. Aldosterone depressed activities of Na^+-K^+-ATPase and Mg^{2+}-ATPase in mesonephros of the 16.5-day-old embryos but did not alter these activities in metanephros. As cholesterol also reduced the same enzyme activities in mesonephros as aldosterone, the decline in Na^+-K^+-ATPase and Mg^{2+}-ATPase may have been nonspecific or toxic reactions to steroids (Table 6A). In the metanephros, aldosterone elevated Ca^{2+}-ATPase and SDH activities and these effects were not duplicated by cholesterol. The stimulation of SDH by the mineralicorticoid was similar to its metabolic actions in other aldosterone-sensitive transporting epithelia in mammals and amphibians (Holmes and Pearce, 1979). Aldosterone treatment did not stimulate Na^+-K^+-ATPase in metanephros of the 16.5-day-old embryo (Table 6B). However, increased Na^+ retention produced by aldosterone in adult birds and mammals (Holmes and Pearce, 1979) can act independently of direct stimulation of the transport enzyme. Rather, increased membrane permeability to Na^+ and/or increased ATP production in response to aldosterone can promote Na^+ reabsorption without directly increasing Na^+-K^+-ATPase activity as measured in homogenates (Holmes and Pearce, 1979). Aldosterone can elevate Na^+-K^+-ATPase activity in the rat kidney, but the specificity of this action has been questioned (Fanestil and Park, 1981).

In summary, few specific conclusions about the mechanisms of action of PRL, ADH, and aldosterone in controlling the distribution of water and ions in the chick embryo and egg can be drawn. Rather, these studies have observed several features of osmoregulatory function to determine if these hormones might act to regulate any aspect of renal output. The evidence presented shows that the kidney of the chick embryo can respond to all three hormones. It remains to be determined whether the embryo actually uses these hormones to protect against excess bodily loss or gain of water when, for example, fluid shifts from allantois to embryo during exposure of incubated eggs to desiccating conditions (Hoyt, 1979). A significant osmoregulatory role in the avian embryo can exist for PRL, since this hormone appears in embryonic blood after Day 13 and reaches peak levels (to 78 ng/ml) between Days 17 and 19 of incubation (Harvey *et al.*, 1979). There is a second peak in circulating PRL after hatching (Harvey *et al.*, 1979). In mammals, PRL seems to have osmoregulatory and developmental roles. It can alter fluid and ion movements across certain fetal membranes (Leontic and Tyson, 1977; Perks *et al.*, 1978) and in fetal skin and bladder (France, 1976; France *et al.*, 1976). Though some results in the fetal mammal, especially in extraembryonic membranes, have been contradictory or have been obtained using ADH-contaminated PRL (cf. Clarke and Bern, 1980), available evidence favors an osmoregulatory role for PRL during gestation. The chick embryo may provide an ideal model system, free from immediate maternal influences, for analysis of the endocrine control of osmoregulatory adjustments made by kidney and other transporting-epithelia during ontogeny.

ACKNOWLEDGMENTS

The authors thank Dr. S. Brown and Dr. P. S. Brown and K. Stocker for a critical reading of this manuscript. We also gladly acknowledge the importance of the writings of Dr. H. A. Bern and C. S. Nicoll in the development of some of the ideas expressed in this paper. Technical help was provided by G. Mohammadzadeh and W. Ling. Ovine prolactin was a gift of the hormone distribution officer, NIAMMD. Supported by Rackham Faculty Grant and by National Science Foundation Grant PCM 7922985.

REFERENCES

Battycharyya, G. K., and Johnson, R. A. (1977). "Statistical Concepts and Methods." Wiley, New York.

Bern, H. A. (1975). On two possible primary activities of prolactins: Osmoregulatory and developmental. *Verh. Dtsch. Zool. Gesellsch.* **1975**, 40–46.

Clark, B., and Porteus, J. W. (1964). Determination of succinic acid by an enzymic method. *Biochem. J.* **93**, 21c–22c.

Clarke, W. C., and Bern, H. A. (1980). Comparative Endocrinology of prolactin. *In* "Hormonal Proteins and Peptides" Vol. 8, (C. H. Li, ed.), pp. 105–197. Academic Press, New York.

Doneen, B. A., and Smith, T. E. (1982). Ontogeny of endocrine control of osmoregulation in chick embryo. I. Role of pituitary gland in distribution of water and ions among embryonic and extraembryonic compartments. *Gen. Comp. Endocrinol.* **48**, 300–309.

Ensor, D. M. (1975). Prolactin and Adaptation. *Symp. Zool. Soc. London* **35**, 129–148.

Fanestil, D. D., and Park, C. K. (1981). Steroid hormones and the kidney. *Ann. Rev. Physiol.* **43**, 637–649.

Fenwick, J. C. (1979). Ca^{++}-activated adenosine triphosphatase activity in the gills of freshwater- and seawater-adapted eels (*Anguilla rostrata*). *Comp. Biochem. Physiol.* **62B**, 67–70.

France, V. M. (1976). Active sodium uptake by the skin of fetal sheep and pigs. *J. Physiol.* **258**, 377–392.

France, V. M., Stanier, M. W., and Wooding, F. B. P. (1976). The effects of hormones and of an osmotic gradient on the structure and properties of mammalian and foetal urinary bladder *in vitro*. *J. Physiol.* **258**, 393–408.

Hardy, M. A. (1978). Intracellular calcium as a modulator of transepithelial permeability to water in frog urinary bladder. *J. Cell Biol.* **76**, 787–791.

Harvey, S., Davidson, T. F., and Chadwick, A. (1979). Ontogeny of growth hormone and prolactin secretion in the domestic fowl (*Gallus domesticus*). *Gen. Comp. Endocrinol.* **39**, 270–273.

Holmes, W. N., and Pearce, R. B. (1979). Hormones and osmoregulation in vertebrates. *In* "Mechanisms of Osmoregulation in Vertebrates" (R. Gilles, ed.), pp. 413–533. Wiley, New York.

Hoyt, D. F. (1979). Osmoregulation by avian embryos: The allantois functions like a toads' bladder. *Physiol. Zool.* **52**, 354–362.

Kinne-Saffran, E., and Kinne, R. (1974). Localization of a calcium-stimulated ATPase in the basal-lateral plasma membranes of the proximal tubule of rat kidney cortex. *J. Membr. Biol.* **17**, 263–274.

Leontic, E. A., and Tyson, J. E. (1977). Prolactin and fetal osmoregulation: Water transport across isolated human amnion. *Amer. J. Physiol.* **232**, R124–R127.

Mainoya, J. R. (1975). Effects of bovine growth hormone, human placental lactogen and ovine prolactin on intestinal fluid and ion transport in the rat. *Endocrinol.* **96**, 1197–1202.

Malarkey, W. B., George, J. M., and Baehler, R. W. (1975). Evidence for vasopressin contamination of ovine and bovine prolactin. *ICRS Med. Sci.* **3**, 291.

North, W. G., Gellai, M., Sokol, H. W., and Adler, R. (1979). Rat prolactin has no intrinsic antidiuretic activity in the rat. Endocrinology Society 61st Annual Meeting, abstr. 881.

Pang, P. K. T., Griffith, R. W., Maetz, J., and Pic, P. (1980). Calcium uptake in fishes. *In* "Epithelial Transport in the Lower Vertebrates" (B. Lahlou, ed.), pp. 121–132. Cambridge Univ. Press, London/New York.

Pahuja, P. N., and deLuca, H. F. (1981). Stimulation of intestinal calcium transport and bone calcium mobilization by prolactin in vitamin D-deficient rats. *Science* **214**, 1038–1039.

Peaker, M. (1979). Control mechanisms in birds. *In* "Mechanisms of Osmoregulation in Animals" (R. Gilles, ed.), pp. 323–348. Wiley, New York.

Perez-Gonzalez de la Manna, M., Proverbio, F., and Whittenbury, G. (1980). ATPases and salt transport in the kidney tubule. *Curr. Top. Membr. Transp.* **13**, 315–335.

Perks, A. M., Vizsolyi, E., Holt, W. S., and Cassin, S. (1978). Hormonal influences on the movement and composition of amniotic fluid. *In* "Comparative Endocrinology" (P. J. Gaillard, and H. H. Boer, eds.), pp. 231–234. Elsevier/North Holland, Amsterdam.

Peterson, G. L. A. (1978). Simplified method for analysis of inorganic phosphate in the presence of interfering substances. *Anal. Biochem.* **84**, 164–172.

Wendelaar Bonga, S. E., and van der Meij, J. C. A. (1980). The effect of ambient calcium on prolactin cell activity and plasma electrolytes in *Sarotherodon mossambicus* (*Tilapia mossambica*). *Gen. Comp. Endocrinol.* **40**, 391–401.

White, B. A., and Nicoll, C. S. (1979). Prolactin receptors in *Rana catesbeiana* during development and metamorphosis. *Science* **204**, 851–853.

White, B. A., and Nicoll, C. S. (1980). Renal and hepatic prolactin receptors among vertebrates. *Amer. Zool.* **20**, abstr. 569.