Effects of Prolactin on Active Sodium Transport Through the Skin of Hypophysectomized Newts[1]

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Abstract

Plasma osmolalities and sodium fluxes across isolated, short circuited sections of abdominal skins were measured in sham-operated and totally hypophysectomized newts *(Notophthalmus viridescens)*. These newts were injected intraperitoneally, each day for 7 days, with an isotonic saline solution either without (control) or with dissolved hormones. The hormone treated groups received one of the following injections: either 25 ug prolactin with 25 ng thyroxine, 25 ug prolactin, 25 ng thyroxine, 25 ug aldosterone, or 115 ng arginine vasotocin. The volume of the injection was 0.05 ml. On the seventh day hypophysectomized newts had significantly lower plasma osmolalities compared to intact and sham-operated newts. Hypophysectomized newts had a significantly higher Na^+ efflux (passive) across the skin with a normal Na^+ influx (active). The increased Na^+ efflux and the decreased plasma osmolality were prevented by exogenous prolactin, particularly when combined with thyroxine. In contrast, neither thyroxine alone, aldosterone, nor arginine vasotocin prevented the increased Na^+ efflux or the decreased plasma osmolality following hypophysectomy. In sham-operated newts both aldosterone and arginine vasotocin increased the active Na^+ influx without altering the Na^+ efflux across the skin. However, neither aldosterone nor arginine vasotocin increased the Na^+ influx in hypophysectomized newts, suggesting a dependency on other pituitary factors. Prolactin influences the permeability to Na^+ at the cutaneous surface; thus it may have an osmoregulatory function in this species of salamander.

Introduction

Removal of the entire pituitary gland interferes with the ability of adult, aquatic newts (*Notophthalmus viridescens*) to survive in water (4, 7). Connelly, Tassava and Thornton (4) demonstrated that prolactin, particularly in combination with thyroxine, prolongs survival of the adult hypophysectomized newts, although thyroxine alone was ineffective. One of their hypotheses was that prolactin might have an osmoregulatory capacity in the newt. The eft water drive, which is stimulated by prolactin, also suggests a possible osmoregulatory influence for prolactin (11). Possible prolactin interactions in sodium and water balance have been studied in several amphibians: in *Triturus cristatus* (15, 19), in *Rana pipiens* and *Taricha torosa* (5), in *Notophthalmus viridescens* (3), in *Xenopus laevis* (21), in *Ambystoma mexicanum* (23, 24), in *Necturus maculosus* (18), and in *Bufo marinus* (6). Conflicting results have been reported and no general relationship between prolactin and osmoregulation in amphibians as a group can be made yet. In most of these studies the researchers elucidated the effects of hypophysectomy and either hormone replacement (started several days after hypophysectomy) or hormone maintenance therapy (started immediately after hypophysectomy) on plasma sodium concentrations. Only in a few studies did the investigators study changes in sodium or water exchange at any of the major sites of exchange. This paper reports the effects of hypophysectomy and hormonal maintenance therapy on sodium fluxes

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through the skin of the adult eastern, spotted newt (*Notophthalmus viridescens*) and on the plasma osmolality.

Materials and Methods

Aquatic, adult newts (*Notophthalmus viridescens*) were obtained from Mogul-Ed, Oshkosh, Wisconsin. They were kept in an aerated, filtered solution containing: 3.8 mM Na^+, 2.9 mM Cl^-, 0.8 mM Ca^{++}, 0.1 mM K^+, and 0.2 mM HCO_3^- at 21-23°C with a pH between 7.0-7.2. Lighting conditions were not controlled. Sham-operated and totally hypophysectomized newts were randomly distributed into seven groups: (1) uninjected; or those which received daily injections for a week of either (2) 0.7% NaCl, (3) 25 μg prolactin, (4) 25 ng thyroxine, (5) 25 μg prolactin with 25 ng thyroxine, (6) 25 μg aldosterone, or (7) 115 ng arginine vasotocin. All hormones were dissolved in isotonic saline in such a concentration that an injection of 0.05 ml of solution delivered the desired amount. All injections were given intraperitoneally to unanesthetized newts through a 30 gauge hypodermic needle passed under the caudal skin into the posterior peritoneal cavity. Aldosterone was purchased from Calbiochem, Los Angeles, California; synthetic arginine vasotocin from Sandoz Pharmaceuticals, Hanover, New Jersey; and thyroxine from Baxter Laboratories, Morton Grove, Illinois. The prolactin (NIH-P-B8) was provided by the NIH Endocrine Study Section, Bethesda, Maryland.

Hypophysectomy was performed by the method of Schotte and Hall (20). Surgical anesthesia was obtained by immersing the newts in 0.3% (w/v) tricaine- methane sulfonate (Kent Chemical Company, Vancouver, British Columbia). After removing a flap of the sphenoid bone directly ventral to the pituitary gland, the entire pituitary was removed by suction through a small glass pipette. Afterwards, the bone flap was replaced. The sham-operated newts were treated similarly except the pituitary gland was not removed. Totally hypophysectomized newts do not molt; in 2-3 days they develop a rough, black skin which is easily distinguished from the smooth, dark green skin of the intact newt (7). This feature can be used as an index of total hypophysectomy (7). Therefore, any hypophysectomized newts, except those treated with thyroxine, which showed signs of molting or failed to develop the characteristic rough, black skin were discarded. Hypophysectomized newts treated with thyroxine retained the normal skin coloration and smoothness; in these newts the adequacy of hypophysectomy was determined by postmortem examination of the brain histologically.

After the newts were treated for 7 days as described above, they were decapitated and a ventricular blood sample was taken. Then the abdominal skin was removed for *in vitro* permeability studies by the short-circuited procedure developed by Ussing and Zerahn (22). With the aid of a dissecting microscope, the musculature was peeled from the inner surface of the skin with care being taken to avoid tearing the skin. The skin was placed in an interlocking ring system which was held between the halves of a lucite bathing chamber containing 4 ml of a buffered amphibian Ringer's solution on each side of the

skin. The pH of the solution was 7.4 ± 0.1, the temperature was 22 ± 2°C and the osmolality was 241-246 mOsm/Kg H_2O. The area of the bathed skin was 2.3 cm^2. After a 30 min equilabration period, 100 μl of a radioactive solution containing 1.0 μCi of $^{22}NaCl$ was added to one side of the chamber. At 1 and at 10 min a 20 μl sample was taken from that same side to check both the completeness and rapidity of mixing of the radioisotope and to check for any leaks in the system (e.g., small holes in the bathed skin). Thereafter, a 20 μl sample was taken from the opposite side (initially unlabelled chamber) for 3 hrs. The osmolality of the plasma was determined by the micromelting point technique of Drucker and Schreiner (8). Analyses of the significance of differences among the treatment and control groups were made using the t-test developed by Dunnett (9).

Results

On the seventh day the saline injected hypophysectomized newts had a lower plasma osmolality than the corresponding group of sham-operated newts (Table 1). This drop in plasma osmolality was prevented by the maintenance therapy with prolactin and thyroxine in combination. Prolactin treatment alone was less effective at the dose used. Thyroxine alone, as well as aldosterone and arginine vasotocine alone, was ineffective. In sham-operated newts the plasma osmolality was not significantly altered by any of the treatments (Table 1).

The effects of hypophysectomy and the various hormonal maintenance therapies on the cutaneous fluxes of sodium, measured under short-circuited conditions *in vitro,* are shown in Table 2. Under short-circuited

TABLE 1. *The effects of hormone treatments on the plasma osmolality of sham-operated and totally hypophysectomized newts. The osmolalities were measured on the seventh day of treatment.*

Group	Treatment	Injection	Dose/newt	Plasma Osmolality mOsm/Kg HOH
(1)	Sham-operated (SO)	None	-----	-------
(2)	SO	Saline	0.05ml 0.7%	242±15
(3)	SO	Prolactin & Thyroxine	25ug+25ng	243±16
(4)	SO	Prolactin	25ug	244±16
(5)	SO	Thyroxine	25ng	242±12
(6)	SO	Aldosterone	25ug	241±14
(7)	SO	Arginine Vasotocin	115ng	238±15
(1)	Hypophysectomized (Hy)	None	-----	-------
(2)	Hy	Saline	0.05ml 0.7%	181±14*
(3)	Hy	Prolactin & Thyroxine	24ug+25ng	231± 6
(4)	Hy	Prolactin	25ug	206±17*
(5)	Hy	Thyroxine	25ng	182±20*
(6)	Hy	Aldosterone	25ug	191±19*
(7)	Hy	Arginine Vasotocin	115ng	180±10*

N=6 newts/group.
Mean ± standard deviation.
* significantly different from sham-operated—saline injected group at = 0.01.

TABLE 2. *The effects of hormone treatments on sodium fluxes across isolated, short-circuited skins of sham-operated and totally hypophysectomized newts. On the seventh day of treatment the fluxes of Na+ were measured* in vitro *under short-circuited conditions using* ^{22}Na.

Group	Treatment	Injection	Dose/newt	Na+ Fluxes uEq/cm²/hr Influx	Efflux	Net Flux
(1)	Sham-operated (SO)	None	-----	0.56±0.13	0.15±0.07	0.42±0.15
(2)	SO	Saline	0.05ml 0.7%	0.60±0.10	0.16±0.04	0.43±0.06
(3)	SO	Prolactin & Thyroxine	25ug+25ng	0.52±0.11	0.14±0.06	0.38±0.10
(4)	SO	Prolactin	25ug	0.52±0.19	0.17±0.04	0.35±0.19
(5)	SO	Thyroxine	25ng	0.53±0.18	0.16±0.08	0.36±0.18
(6)	SO	Aldosterone	25ug	0.92±0.16*	0.15±0.06	0.84±0.22*
(7)	SO	Arginine Vasotocin	115ng	0.85±0.13*	0.14±0.07	0.71±0.12*
(1)	Hypophysectomized (Hy)	None	-----	0.62±0.11	0.47±0.07*	0.15±0.07*
(2)	Hy	Saline	0.05ml 0.7%	0.63±0.13	0.50±0.09*	0.13±0.10*
(3)	Hy	Prolactin & Thyroxine	25ug+25ng	0.58±0.07	0.20±0.06	0.38±0.12
(4)	Hy	Prolactin	25ug	0.55±0.07	0.26±0.06	0.30±0.08*
(5)	Hy	Thyroxine	25ng	0.64±0.10	0.42±0.09*	0.22±0.06*
(6)	Hy	Aldosterone	25ug	0.64±0.06	0.41±0.06*	0.23±0.05*
(7)	Hy	Arginine Vasotocin	115ng	0.64±0.09	0.47±0.11*	0.17±0.05*

N = 10 newts per group.
Mean ± standard deviation.
* significantly different from sham-operated—saline injected group at $\alpha = 0.01$.

conditions *in vitro*, sodium is actively transported inward across the skin of the newt and passively diffused outward across the skin; the net flux of sodium equals the measured short-circuit current across the skin (10). Although hypophysectomy did not alter the active Na+ influx, there was an increased passive Na+ efflux following hypophysectomy. This increased Na+ efflux in the hypophysectomized newt skin was prevented either by prolactin alone or by prolactin in combination with thyroxine. In contrast, treatment with thyroxine alone, aldosterone or arginine vasotocin did not reduce or prevent this increased Na+ efflux. In the sham-operated newts aldosterone and arginine vasotocine increased the active Na+ influx without altering the passive Na+ efflux. But this effect was not observed in the hypophysectomized newts on the seventh day. Exogenous prolactin, either alone or in combination with thyroxine, did not decrease Na+ efflux in the sham-operated newts, as was seen in the hypophysectomized newts.

Discussion

Following hypophysectomy the plasma osmolality decreased significantly. Maintenance treatments with prolactin alone partially prevented this decrease, but a combined treatment with prolactin and thyroxine was more effective. This relates to the finding of Connelly *et al.* (4) that a prolactin-thyroxine treatment promoted survival of

the aquatic adults better than a prolactin treatment alone. Death following hypophysectomy is probably due to an inability to regulate water and electrolyte balance. Brown and Brown (3) reported that following hypophysectomy in *Notophthalmus viridescens* plasma Na^+ concentrations decreased. They reported, however, that either prolactin alone or corticosterone alone partially reverses the decreased plasma Na^+ concentrations. However, there was no apparent synergism between prolactin and corticosterone when administered together. In other amphibians plasma Na^+ concentrations also fall after hypophysectomy or adenohypophysectomy (5, 16, 18, 19, 23, 24). But differences in the effectiveness of hormones have been reported. In *Necturus maculosus* a prolactin treatment started on the sixteenth day after hypophysectomy only partially corrected the fall in plasma Na^+; whereas, a prolactin treatment started on the same day of hypophysectomy completely prevented the hyponatremia by somehow decreasing the Na^+ efflux across one or more of the membranes of exchange with the external environment (18). In *Triturus cristatus* either prolactin or aldosterone corrected the hyponatremia following hypophysectomy (19). Adenohypophysectomy of *Bufo marinus* causes a progressive hyponatremia, which can be corrected with glucocorticoids (cortisol or corticosterone) but not with aldosterone (16). In adenohypophysectomized *Rana pipiens* prolactin did not correct the hyponatremia (5). In adenohypophysectomized *Taricha torosa* neither prolactin alone nor aldosterone alone restored plasma Na^+ levels to normal, but when given together they partially corrected plasma Na^+ concentrations (5). In hypophysectomized, larval *Ambystoma mexicanum* prolactin is an effective hormone (23, 24). It is hard to explain these apparent inconsistencies at this time; but, differences in dosages, treatment schedules, and physiological aspects (e.g., aquatic dependency of the species) may be involved.

The hyponatremia following hypophysectomy may not be due entirely to a loss of Na^+ to the environment. Water balance and water turnover rate could be involved. In adenohypophysectomized *Rana pipiens* cutaneous permeability to water is increased (12, 13). This could lead to a diluting effect on plasma components. In premetamorphic larvae of *Xenopus laevis* the turnover rate of water is depressed by hypophysectomy and is restored to normal by prolactin (21). Redistribution of Na^+ within the various compartments of the body is also a possibility.

There are fewer studies on the possible sites of action of prolactin. Wittouck (24) reported that the isolated gills from hypophysectomized, larval *Ambystoma mexicanum* absorbed less Na^+ from a NaCl solution than the isolated gills from intact animals; prolactin enhanced absorption of Na^+ in both groups. Adenohypophysectomy of *Rana pipiens* increases the passive efflux of Na^+ and decreases the active transport of Na^+ (influx) across the skin; these changes are partially prevented by ACTH or aldosterone (17). In adenohypophysectomized *Bufo marinus* urine flow, urine osmolality, and urine Na^+ concentration were not significantly different from the sham-operated, intact toads (16). Dalton & Snart (6) reported a stimulatory action of exogenous prolactin on the active transport of Na^+ by the isolated, short-circuited bladder of

Bufo marinus; but, a similar effect was not shown in the isolated short-circuited skin of *Bufo marinus* or *Rana pipiens*. Prolactin stimulated the active transport of Na^+ by increasing the passive permeability to Na^+ at the mucosal surface of the urinary bladder (this allows more Na^+ to diffuse inward to the site of the transport carrier molecules) (6). In the present study prolactin, particularly in combination with thyroxine, decreased the passive permeability to Na^+ (efflux) in the skin of hypophysectomized newts. Active Na^+ influx was not altered by hypophysectomy or by prolactin. This would aid in a positive Na^+ balance. The mechanism of action is not known, but perhaps prolactin influences the passive permeability to Na^+ by stimulating the production of mucus (26). In the adult newt the kidneys and urinary bladder are other possible sites of action for prolactin. Prolactin did not reduce the passive cutaneous permeability to Na^+ in the intact newts, although it had this effect in the hypophysectomized newts. Possibly the endogenous secretion of prolactin was near maximal levels, thus the exogenous prolactin would not have produced a significant change in permeability to be detected. A similar lack of a detectable response to prolactin in intact axolotls has been observed (25). If prolactin stimulates mucus secretion, then it might also be possible that the exogenous prolactin might not have been sufficient to increase significantly the mucus layer on the skin and thus detectably alter the permeability to Na^+.

Although aldosterone and arginine vasotocin were not effective in correcting the hyponatremia in hypophysectomized newts, they did stimulate independently the active transport of Na^+ in the sham-operated, intact newts, but not in the hypophysectomized newts. Because they were effective in the intact newts, tachyphylaxis can be ruled out as an explanation for the lack of response in the hypophysectomized newts. Some pituitary factor or pituitary dependent factor is necessary before either aldosterone or arginine vasotocin can stimulate the cutaneous active transport of Na^+ in the newt. In a separate study (unpublished) arginine vasotocin effectively stimulated active Na^+ transport in the newt skin 4 hrs after hypophysectomy (when it is likely that the necessary factor was still present or the target tissue was still affected by the factor previously secreted) but not after 4 days (when the necessary factor would probably have been metabolized). Refractoriness to posterior pituitary hormones, which normally increase the cutaneous permeability to water in anurans, has been reported in adenohypophysectomized frogs by Jones and Steggarda (12) and by Levinsky and Sawyer (13). The refractoriness to exogenous antidiuretic hormone could be prevented by administering both ACTH and thyroxine together (13). Stimulatory effects of aldosterone and ADH on Na^+ transport have been reported in anurans and urodeles (1, 2, 14).

Prolactin is involved in the electrolyte balance of the newt, *Notophthalmus viridescens*, because it influences the cutaneous permeability to Na^+. Whether this is a direct action or an indirect action is still unknown. Similar studies should be done in the larval and eft stages of this newt. Perhaps this could give a better insight into the

migration to a semiterrestrial habitat after metamorphosis and the subsequent migration back to water (second "metamorphosis") that is characteristic of this and related newts.

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