Effect of 2-Br-α-Ergocryptine on Fresh Water Survival in the Teleosts, *Xiphophorus hellerii* and *Poecilia latipinna*

It is well established that various euryhaline species of teleosts require prolactin in order to survive in fresh water (see BALL[1]). The physiological role that prolactin plays in one of these fish, *Poecilia latipinna*, is to conserve plasma sodium when the fish is in a hypotonic medium (BALL and ENSOR[2]). Evidence in this field has largely been obtained by experiments involving hypophysectomy followed by replacement therapy with prolactin. Hypophysectomy is difficult and often not possible with certain teleosts. A more simplified alternative method to remove endogenous circulating prolactin would therefore be advantageous to investigate possible osmoregulatory functions of prolactin in other teleosts. There have been several reports that an ergot alkaloid, 2-Br-α-ergocryptine-methane-sulfonate (CB 154), inhibits the secretion of pituitary prolactin in several mammals (FLÜCKIGER and WAGNER[3], HEUSON et al.[4], YANAI and NAGASAWA[5], BILLETER and FLÜCKIGER[6], CASSELL et al.[7], LUTTERBECK et al.[8], PASTEELS et al.[9], and STÄHELIN et al.[10]). This investigation was undertaken to determine if this compound could also inhibit prolactin secretion in two teleosts, *Xiphophorus hellerii* and *Poecilia latipinna*.

Materials and methods. The fish used for this investigation were adult *X. Hellerii* (1.14 ± 0.12 g) and *P. latipinna* (5.15 ± 0.26 g). All fish were maintained in 20 gal glass aquaria, at approximately 27 °C, on a natural photoperiod, and fed once per day with a commercial fish food. Prior to any experimentation, fish were acclimated to dilute salt water (12% commercial sea salts) for 7 days.

In the first experiment 1 group of 7 *X. hellerii*, which were maintained in dilute salt water, were injected i.p. each morning with 10 µl of a freshly prepared solution containing 10% ethyl alcohol in 0.10 *M* NaCl and 5.0 mg/kg body weight each of CB 154 and tartaric acid. A second group of 7 fish were given the same injections but minus CB 154 (vehicle injection). After 1 week all fish were transferred to fresh water and the injections continued until all the CB 154-injected fish died.

A second experiment was conducted exactly as the first but 2 groups of 15 *P. latipinna* were used instead. Also, the injection volume was increased to 25 µl. The pituitary glands from all injected fish were dissected out and fixed in Bouin Hollande Sublimate (HERLANT[11]). The glands were later embedded in paraffin, sectioned at 5 µm, and stained with either HERLANT's tetrachrome (HERLANT[12]) or Masson's trichrome (CULLING[13]). The intensity of staining of the *eta* cells of the CB 154- and vehicle-injected fish was compared. Also, the *eta* cellular and nuclear diameters were compared.

The effect of CB 154 concentrations on plasma sodium levels was investigated by using 34 *P. latipinna* divided into 4 groups. Groups 1–3 received injections as previously described with concentrations of CB 154 of 10 ($N = 10$), 5 ($N = 10$), and 2.5 ($N = 10$) mg/kg body weight. Group 4 ($N = 4$) served as uninjected controls. Again, the fish were injected for 1 week while in dilute salt water (12%) and then transferred to fresh water. Blood samples were taken by heart puncture with a microsyringe when the fish failed in fresh water or 1 week after transfer, which ever came first. The blood was centrifuged in a heparinized

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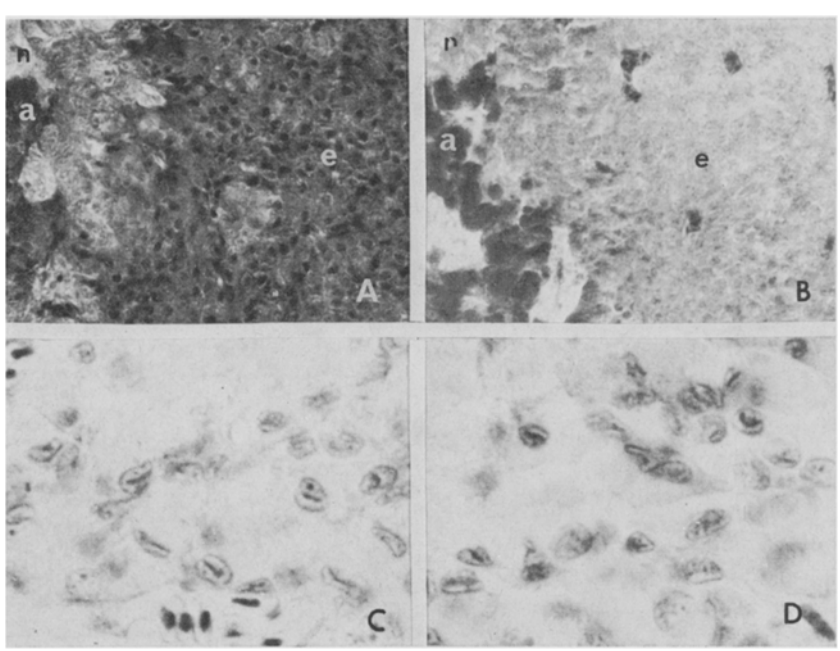

Sagittal sections of the pituitary of *P. latipinna* stained with Mason's trichrome showing: acidophil cells of the proximal pars distalis (a), pars nervosa (n), and *eta* cells of the rostral pars distalis (e). A) Fish injected with CB 154 for 7 days in dilute salt water and 7 days in fresh water. Note dark cytoplasmic staining of *eta* cells. ×270. B) Fish injected with vehicle for 7 days in dilute salt water and 9 days in fresh water. Note lesser cytoplasmic staining of the *eta* cells. ×270. C) and D) *Eta* cells in fish injected with CB 154 for 7 days in dilute salt water and 7 days in fresh water. Note many folded nuclei. ×1110.

microhematocrit tube and the plasma then diluted 1:400 in 0.1 *N* HCl. Sodium concentrations in this diluted plasma were then measured on a Zeiss PMQ II flame photometer.

Results. The number of days that *X. hellerii* survived in fresh water after CB 154 injections ranged from 1–15 days. The average survival time was 9.3 ± 2.2 days ($\bar{X}$ = S.E.M.). One fish injected with CB 154 died prior to transfer to fresh water. No vehicle-injected fish died throughout the experiment.

When the CB 154-injected *P. latipinna* were transferred to fresh water they survived from 1–9 days with an average of 4.9 ± 1.0 days. One fish injected with CB 154 and 2 vehicle-injected fish died before transfer to fresh water. No vehicle-injected fish died in fresh water.

There was no significant difference apparent in the *eta* cells of the pituitary glands of the two groups of *P. latipinna* with respect to cell size, nucleus size, or the ratio cell size/nucleus size. However, the cytoplasmic granulation of the *eta* cells in the CB 154-injected fish appeared to exhibit a stronger stainability than in the vehicle-injected fish (Figure). Also, the nucleus more frequently had a folded appearance and the nucleoli were less pronounced in the CB 154-injected fish.

There were no differences between the plasma sodium concentrations in the uninjected control fish and fish that received injections of 2.5 mg CB 154 per kg body weight. These 2 groups, however, had higher plasma sodium concentrations than those groups that had injections of CB 154 in concentrations of 5 or 10 mg/kg body weight ($p < 0.05$) (see Table).

Discussion. The failure of hypophysectomized *P. latipinna* in fresh water has been shown to be due specifically to the lack of prolactin (BALL and OLIVEREAU[14]). Thus, it appears that CB 154 has the same effect as hypophysectomy with respect to the available circulating supply of this hormone. The average survival time of 9.3 days for *X. hellerii* is in close agreement with 8.6 days reported by SCHREIBMAN and KALLMAN[15]. However, the average survival time of 4.9 days for *P. latipinna* is high compared to the results of 1–2 days reported by BALL and OLIVEREAU[14]. Whether this is an effect of CB 154 or a different race of fish is not known.

As previously mentioned, there are a number of investigations which indicate that CB 154 inhibits the secretion of prolactin in several mammals. Further, it has been shown by PASTEELS et al.[9] that the secretion of prolactin from rat and human hypophyses in organ culture can be inhibited by CB 154. They thus suggest that CB 154 inhibits the exocytosis of secretory granules by direct action on the prolactin cells. This may also be the case in *P. latipinna* since these fish injected with this same compound appeared to have *eta* cells of the pituitary gland which were more granulated and appeared less active than the *eta* cells of fish injected with the vehicle alone. The *eta* cells are most likely the pituitary source of teleost prolactin (see BALL and BAKER[16]).

BALL and ENSOR[2] have proposed that the reason that hypophysectomized *P. latipinna* fail in fresh water is due to a drop in plasma sodium. This drop in plasma sodium can be specifically prevented by ovine prolactin. This more specific physiological parameter which exists in the absence of prolactin was also evident after CB 154 injections in concentrations of 5 and 10 mg/kg body weight. Injections of 2.5 mg CB 154 per kg body weight were ineffective in lowering the plasma sodium levels. Thus, in appropriate concentrations, CB 154 prevents the maintenance of a normal plasma sodium concentration which is specifically controlled by prolactin.

The action of CB 154 appears to be similar in *X. hellerii* and *P. latipinna* as in several mammals. Whether CB 154 specifically inhibits the release of prolactin but has no effect on the release of any other hormone in these two fish is unknown. If it is a specific action on prolactin release only, then CB 154 could be a most useful compound in investigations concerning prolactin physiology in other species of teleosts.

Zusammenfassung. Die Injektion von 2-Br-α-ergokryptin (CB 154) hemmte das Überleben von *Xiphophorus helleri* und *Poecilia latipinna* im Süsswasser. CB 154 senkte die Plasma-Na-Konzentration von *P. latipinna*, was als Zeichen einer Hemmung der Prolaktinsekretion interpretiert werden kann. Auch erschienen die wahrscheinlich Prolaktin sezernierenden *aeta*-Zellen der Hypophyse inaktiv nach CB 154. Es ist noch unbekannt, ob CB 154 direkt oder indirekt die Prolaktinsekretion der Hypophyse hemmt.

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Effect of CB 154 on plasma sodium concentrations in *P. latipinna* in freshwater

Treatment	Sodium concentration (mEq/l)[a]
1. No injection	159.3± 1.6
2. 2.5 mg CB 154/kg body weight	166.6±11.0
3. 5.0 mg CB 154/kg body weight	127.2± 9.4
4. 10 mg CB 154/kg body weight	130.7± 8.5

[a] Mean±S.E. Sodium in freshwater 3.98 mEq/l.

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Neurosecretion in Ehrlich Ascites Carcinoma-Bearing Mice

The dependence of tumours of endocrine and target organs on the internal hormonal environment, as well as the production of such tumours by a hormonal imbalance, is widely recognized as important in human and animal carcinogenesis[1]. Some hormones may act as carcinogens or promote the growth of human and experimental tumours[1–3]. The role of neurohormones also appears to be significant in this respect. It has been shown, for example,