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Severn Borden Churn

Rapid Increase in Serum Calcium in Response to Stress in *Pomoxis nigromaculatus*, Black Crappie

by

Severn Borden Churn

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Severn Borden Churn

Approved:

William S. Woolcott
Committee Director

Committee Director

Examining Faculty:

R Deandecker

F. B. Lotspeich

W. John Hayden

W. R. West Jr.

Melanie L. Hopkins

John W. Finley

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Abstract

Serum calcium levels were determined colorimetrically in Pomoxis nigromaculatus, the black crappie, subjected to rapid changes in temperature. Two experiments were conducted; in the first, fish were exposed to stress for 30 minutes before measuring serum calcium, and in the second, fish were exposed to stress for 5 minutes and returned to normal conditions for 25 minutes before measuring serum calcium. Condition (K-value) and sexual development were noted for the fish. Serum calcium increased in all stressed fish, regardless of sex, but higher serum calcium values were observed in stressed mature females than in stressed males or stressed immature females-no difference was observed for unstressed control fish. The initial temperature change appears to be the stimulus for the rapid rise in serum calcium as neither the direction of the temperature change nor the duration of the stress affected the results. It is suggested the source of the calcium observed in the present study is internal and probably mobilized from scales.

Introduction

Calcium is commonly known to participate in the reaction of mammals, including man, to prolonged stress. Recently, Cobb (1985) has shown that calcium mobilization in response to stress occurs within 90 minutes, which is much faster than previously thought. The objective of the present study was to determine whether fishes, which are phylogenetically distant from mammals, respond to stress by mobilizing calcium and if the mobilization occurs rapidly as has been shown in mammals.

Methods and Materials

Pomoxis nigromaculatus, the black crappie, were collected in Lake Anna and Virginia Power's North Anna Power Plant waste-heat treatment facility in Louisa County, Virginia by direct current electrofishing (courtesy Virginia Power Environmental Laboratory) on four collecting trips in the winter and spring of 1986. The fish were transported to the University of Richmond in a battery aerated, 30 gal, styrofoam cooler. In the laboratory, fish were kept in a 300 gal storage tank, filtered by three Dynaflow 150 pumps, for at least a week before experimentation to allow for acclimation by the fish to the laboratory environment. Pomoxis nigromaculatus were fed feeder fishes (from a local pet store) and minnows (Cyprinidae) seined from nearby tributaries of the James River. Temperature (17-20C) and pH (7.8-8.1) of the tank water were closely monitored. Fungal or bacterial infection

were controlled by adding methylene blue (5% solution, 1 drop/gal water) or formalin (15% solution, 1 drop/gal water) to the tank.

Two experiments were done. The experimental design for the first experiment was as follows.

Day 1. Two 30 gal tanks were set up in the same room that contained the storage tank. Chemically dechlorinated water (Genesis, Aquarium Products) of the same pH as the storage tank was added and equilibrated to ambient temperature. A charcoal filter and air stone were used for filtration.

Day 2. Two fish were selected at random from the storage tank, transferred to the experimental tanks (one/tank) and allowed to acclimate to the new tank for 24 hours.

Day 3. Water (approximately 20 gal) was removed from each tank until the dorsal fins of the fish were above the surface of the water. New, dechlorinated water was added to each tank as follows:

Tank 1. 20 gal water of the acclimated temperature

Tank 2. 20 gal water 10 C colder than acclimated temperature

Thirty minutes after the initial exposure to stress fish were removed from the water, and a ventral incision was made in each fish exposing the heart. Blood was collected into a 1 cc tuberculin syringe from the ventral aorta, transferred to a plastic test tube (Kimball 12 x 75) and refrigerated for 1 hour. During this time, length and weight measurements of the fish were taken for subsequent K-value (weight E5/total length^3) (Car-

lander, 1977) determination. Sex was noted, gonads removed, weighed and percentage body weights recorded.

After clotting, the blood was centrifuged at 3000 rpm for 20 minutes to separate serum from the other blood components. Serum was removed and calcium concentration determined colorimetrically with a calcium diagnostic kit (Sigma Chemical Co. St. Louis, MO: procedure 586). Only plastic containers and pipettes and nanopure deionized water were used to reduce calcium contamination.

In the preliminary experiment, it was found that interpretable data could not be obtained from fish exposed to 10 C warmer water for 30 minutes due to high mortality. Therefore, a second experiment, hot-shock/cold-shock, was done. The experimental design for the second experiment was the same as for the first experiment except as follows.

Day 1. Six 30 gal tanks (3 experimental; 3 acclimation) were set up in the same room that contained the storage tank.

Day 2. Three fish were selected at random from the storage tank, transferred to the experimental tanks (one/tank) and allowed to acclimate to the new tank for 24 hours.

Day 3. Water (approximately 20 gal) was removed from three of the tanks until the dorsal fins of the fish were above the surface of the water. New, dechlorinated water was added to each tank as follows:

Tank 1. 20 gal water of acclimated temperature

Tank 2. 20 gal water 10 C colder than acclimated temperature

Tank 3. 20 gal water 10 c warmer than acclimated temperature

Five minutes after initial exposure to stress fish were transferred to the tanks containing water of acclimated temperature. Thirty minutes after initial exposure to stress, the fish were removed from the water and blood samples and data were obtained as described for experiment one.

Differences in serum calcium levels for all groups were analyzed using Bartlett's Homogeneity Test (Zar, 1974). Results were parametric and were tested for significance using Student's t test and analysis of variance. Data were analyzed for whole populations and, separately, according to sex. Data for calcium levels and condition (K-values) of the fish were correlated using Spearmann Rank Test (Zar, 1974).

Results

In the first experiment Pomoxis nigromaculatus subjected to cooler water than was in the control tank showed increased serum calcium levels of approximately 23% (Fig. 1A). In the second experiment P. nigromaculatus subjected to cold- or hot-shock showed overall increased serum calcium levels of 23% and 26.5%, respectively (Fig. 1B). All fish were in good condition with K-values that approximated those found in the literature for the species (Carlander, 1977).

When data were reorganized to examine differences in serum calcium according to sex, stressed mature females exhibited 8.3% higher serum calcium than stressed males or stressed immature females (Fig. 2). There was no difference in serum calcium levels between stressed males and stressed immature females, nor was there any difference between unstressed males and unstressed females.

Serum calcium values for the stressed groups (cold, heat-shocked, and cold-shocked) were not significantly different from each other. The values of the stressed groups for both experiments were combined and rearranged according to sex (Fig. 2). Data for stressed females showed differences between mature and immature fish and are reported individually. Data for stressed mature and immature males when re-analyzed showed no difference and are reported together. The data for unstressed control males and females showed no differences and were pooled, respectively.

Discussion

The evidence supports temperature change as the stimulus for the rise in serum calcium, and not the actual temperature, because a similar increase in serum calcium occurred in both hot and cold experiments. Furthermore, the stimulus appeared to be the initial change because a similar increase ocurred whether fish were removed from the source of the stress after 5 minutes, as they were in the cold-shock/hot-shock experiment, or allowed to remain in the experimental tank the entire 30 minutes.

The serum calcium increase occurred in all stressed fish

regardless of sex, but mature females were more sensitive to stress than were males and immature females. This increased sensitivity is apparently influenced by ovarian development, although this does not appear to influence the calcium values in unstressed female fish because they were the same as unstressed male fish.

The evidence also points to scales as the probable source of the rapidly mobilized calcium observed in this experiment. Under normal conditions, fish acquire calcium from the environment via their gills and skin (Taylor, 1985; Fleming, 1974), but this mechanism is too slow (Parsons et al., 1978) to be the primary method of rapid serum increase, and therefore, fish must tap an internal source. However, the skeleton of higher teleosts, e.g. P. nigromaculatus, lack the cellular bone present in mammalian skeletons. Although calcium turnover occurs in such acellular bone tissue (Fleming 1974), rapid mobilization is unlikely because acellular bone is not capable of undergoing massive bone remodeling (Taylor, 1985; Moss, 1963). However, fish scales possess a morphology that is similar to membrane derived bone in mammals (Waterman, 1970; Wallin, 1953), and are bordered by osteoclastic-like and osteoblastic-like cells on their periphery (Simkiss, 1973; Waterman, 1970). In fish, calcium turnover rates in scales are approximately three times that of acellular skeletal bone (Fleming, 1974) and scale resorption in response to starvation is well documented (Simmons, 1971; Crichton, 1935). Thus, scales represent the most logical source of rapidly

mobilizable calcium in fish.

The present study demonstrates that the rapid movement of calcium into the blood during stress is not restricted to mammals but also occurs in fish and thus appears to be conserved in the evolutionary process. Also, this study advances a new, readily accessible, external bone model for studying calcium mobilization in response to stress.

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Fig. 1. Serum calcium increase in response to thermal stress. A. 30 min cold group is significantly different from control at $p < 0.025$. B. 5 min cold is significantly different from control at $p < 0.025$, 5 min hot is significantly different from control at $p < 0.001$. All stressed groups are not different from one another at @=0.1. Thin bar represents standarderror of the mean.

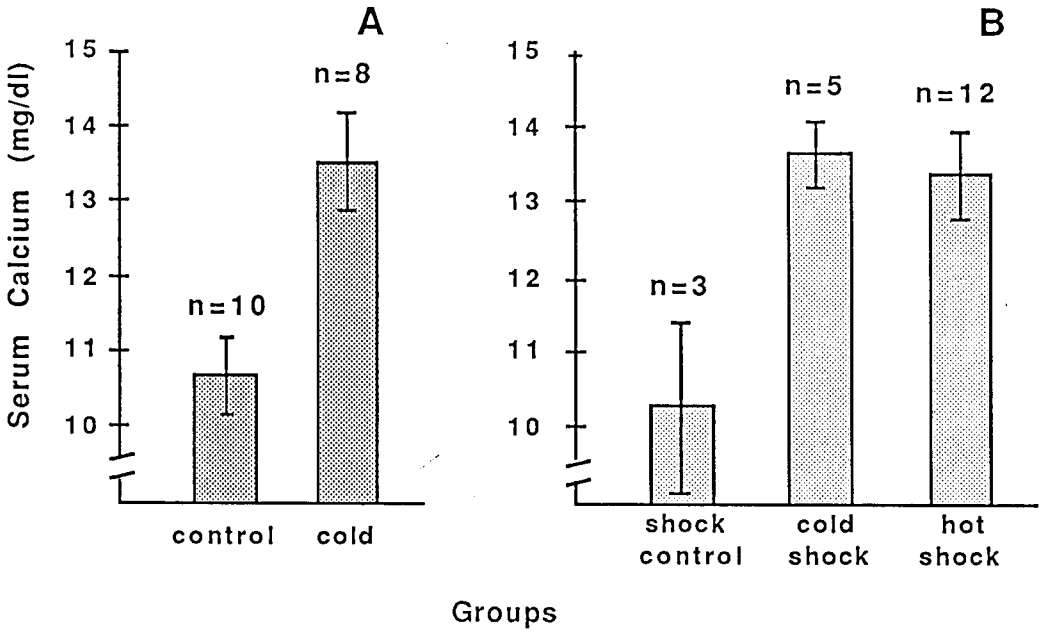
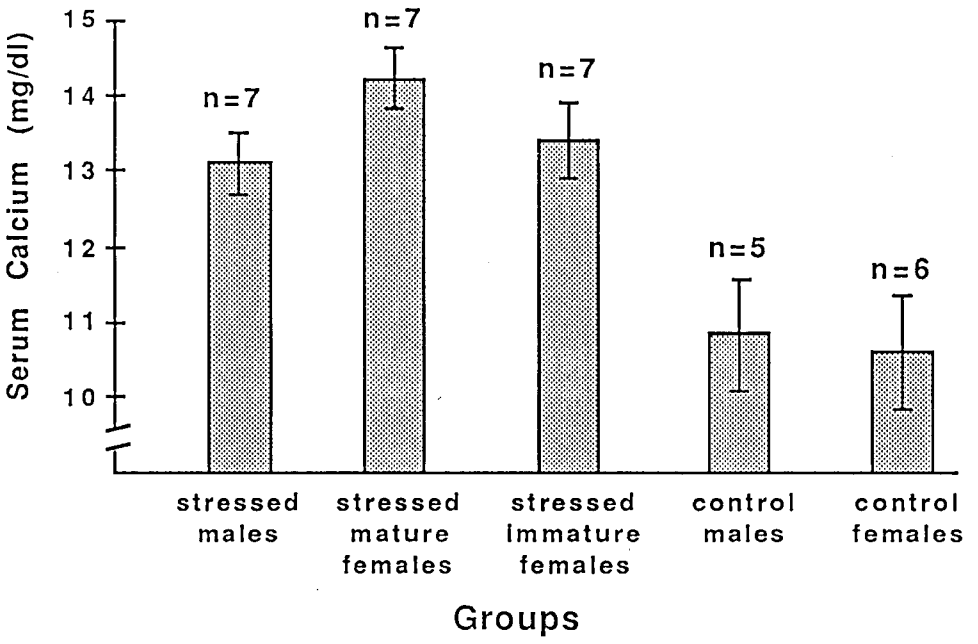

A
B
Serum Calcium (mg/dl)
15
14
13
12
11
10
n=10
n=8
n=3
n=5
n=12
control
cold
shock control
cold shock
hot shock
Groups

Fig. 2. Serum calcium rearranged according to sex. Stressed mature females are significantly different from stressed males and stressed immature females at $p < 0.025$. Stressed males are not different from stressed immature females at @=0.1. All stressed groups are significantly different from controls at $p < 0.001$. Sex data was not obtained for two control and four stressed fish. Thin bar represents standard error of the mean.


Serum Calcium (mg/dl)
15
14
13
12
11
10
n=7
n=7
n=7
n=5
n=6
stressed males
stressed mature females
stressed immature females
control males
control females
Groups

Appendix

Calcium is necessary in every living cell. In plants critical amounts of calcium are required for correct cell ultra-structure, flowering, nodulation of legumes and proper root growth. Animals require calcium for maintenance of cell membranes, electrical propagation, as a cofactor for extracellular enzymes and as an intracellular regulator in cyclosis and muscle contraction.

Calcium, because of its importance, is one of the most regulated minerals in vertebrates (Copp, 1970). In the simplest form, mammals maintain proper blood calcium levels by calcium mobilization from bone. The mobilization is stimulated by parathyroid hormone (PTH), secreted by the parathyroid gland, and inhibited by calcitonin, secreted by cells in the thyroid gland.

Fish lack a parathyroid gland, and presumably a source of PTH. The pituitary gland has been investigated to isolate a hypercalcemic factor that replaces PTH. Early work with killifish Fundulus heteroclitus and other Fundulus species pointed to prolactin as the hormone that assumes PTH's role in fishes (Lam, 1972; Pang et al., 1971). Later work with eels (Anguilla rostrata), which are able to survive hypophysectomy, demonstrate prolactin is a general hydromineral regulating hormone in fishes and is not specific for calcium (Johnson, 1973).

ACTH has been shown to be a hypercalcemic factor in fishes but like prolactin, ACTH is an overall hydromineral regulator.

Unlike prolactin, ACTH seems to be more important in marine fishes whereas prolactin appears to be more important in freshwater fishes (Johnson, 1973).

Peter Pang (1973), using pituitary extract replacement therapy, tried to isolate the pituitary hypercalcemic factor. By segregating the pituitary into section (anterior, median, and posterior) he was able to narrow the hypercalcemic factors to the anterior and posterior sections. Prolactin and ACTH were shown to be the hormones capable of acting as hypercalcemic factors from the anterior portion. However, in the posterior portion, which corresponds to the pars intermedialis in mammals, Pang was unable to isolate the involved hypercalcemic factor.

Albert Parsons et al. (1978) isolated a pituitary factor from fishes immunologically related to PTH isolated from mammals that retain the pars intermedialis. This work looks promising, however, no one has repeated the findings.

Because fish live in a fluid medium they are able to acquire calcium directly from their environment, a feat impossible for land vertebrates (Taylor, 1985). Calcium concentrations in different waters around the world have led fish to develope complex methods of regulating their internal calcium concentration, and this mechanism may vary from species to species or in some cases within species (Taylor, 1985; Pang, 1971). For this reason, locating the overall regulators of blood calcium level in fishes will be very difficult.

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Vita

Severn B. Churn was born in Hopewell, VA on December 7, 1961. He attended Bruton High School in Williamsburg, VA and graduated in June, 1980. He attended The College of William and Mary where he graduated with a B.S. in Biology in May, 1984. He studied at the University of Richmond and will graduate with a Masters of Science in Biology in August of 1986, after which he will attend the Medical College of Virginia, studying for his Doctorate in the Department of Pharmacology.