PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 157, 665–668 (1978)

Renin and Aldosterone Levels in Dolphins and Sea Lions (40117)[1]

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Marine cetaceans are unique in the mammalian world in that most species are never exposed to fresh water. However, little attention has been directed at ascertaining the physiological control mechanisms in the regulation of water balance in these animals. Although some animals may drink small volumes of sea water, their predominant source of water is their food. A similar situation exists regarding salt intake. Thus there is little chance to vary the ratio of salt to water in their diet. What then, are the control mechanisms responsible for maintaining the salt and water balance in these marine animals?

It has been shown that marine cetaceans have measurable circulating levels of renin (1) and urinary aldosterone (2), although no demonstrable levels of ADH have been found (3). The presence of both renin and aldosterone is consistent with the hypothesis that these hormones are involved in the regulation of salt and water balance. To date, there has been no information regarding variations in the levels of either of these hormones with the rate of excretion of Na or, indeed, with diet. There is also no evidence that the renin-angiotensin system stimulates aldosterone secretion in marine cetaceans and pinnipeds.

If the renin–angiotensin system is a stimulator aldosterone in these animals, as it is in most other mammals, one would expect the two hormones to vary together as the diet of the animals is altered. In addition, if these hormones play some role in the regulation of Na excretion, one would expect a negative relation between the rate of sodium excretion, or urinary concentration of Na, and the plasma levels of both renin and aldosterone (4, 5). Accordingly, the following experiments were done to determine if such a relation exists in these animals.

Methods. The dolphins were adult female *Tursiops* sp. maintained in salt water pools at Sea World in San Diego, California since their collection in the Gulf of California near San Felipe, Mexico in the mid 1960's. The dolphins were all sexually mature, around 3 mm in length and maintaining weights of 300 ± 50 kg during the year prior to the study. Two different types of experiments were done. First, the animals were fed each morning at the same time. On day one the animals were fed their normal fish diet and a blood sample was collected 3 hr later. To do this the pool was drained, the animals were placed on a foam rubber pad and kept moist while the sample was collected from the tail fluke (2). Blood sampling required only a few minutes after which the water was raised to its normal level. Salt was fed to the animals in gelatin capsules that were placed in their food fish. Blood samples were collected at the same time each day, before, during and after the salt feeding days and fasting days.

In the second type of experiment two of the dolphins were taken from the pool and laid on a foam rubber mattress. A urinary catheter with inflatable cuff was placed in the urethra for collection of all urine for analysis and volume measurements. Blood samples were taken at the start and hourly for 8 h. Urine volume was measured hourly and an aliquot kept for analysis. Four to six liters of fresh water were given by stomach tube about one hour after the first blood sample was taken. The experiment was done in April and again in October employing the same two animals.

All blood was collected in heparinized syringes and immediately centrifuged. The plasma was separated and frozen for analysis. The sea lions were 3–6 years old males, none of which had reached sexual maturity. All animals had been acquired in a sick condition from southern California beaches and rehabilitated at least 1 year prior to the experiment. Two of the sea lions were maintained in fresh water pools and two in salt water pools at the Marine Life Sciences Laboratory

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[1] This work was supported by NIH Grant No. AMO-5077.

0037-9727/78/1574-0665$01.00/0

of the Naval Undersea Center in San Diego. No differences in hormone levels were noted between the two groups.

Plasma renin activity (PRA) and concentrations of aldosterone were estimated using radioimmunoassay methods. PRA was determined by a modification of the method of Haber *et al.* (6). Samples were incubated for 20 and 40 min to insure linear generation of angiotensin I. Aldosterone was measured using the New England Nuclear Radioimmunoassay Pak (New England Nuclear, Worcester, MA). All samples were done in quadruplicate. Na was determined by flame photometry. Creatinine was measured by the method of Bonsnes and Taussky (8).

Results. Figure 1 shows the results of a single experiment in which plasma from a female dolphin (*Tursiops* sp.) was incubated in the absence and in the presence of additional dog substrate, or with the addition of hog renin. These data show that generation of angiotensin was linear with time, up to approximately 60 min and then fell off. The addition of substrate to the plasma had no effect on the generation rate of angiotensin, indicating, that in the experiments reported here, substrate was not limiting, the PRA values are a good measure of the renin concentration in the samples. If hog renin was added to the plasma, the generation rate of angiotensin was also linear for 60 min, but each point was considerably above that obtained without the addition of hog renin.

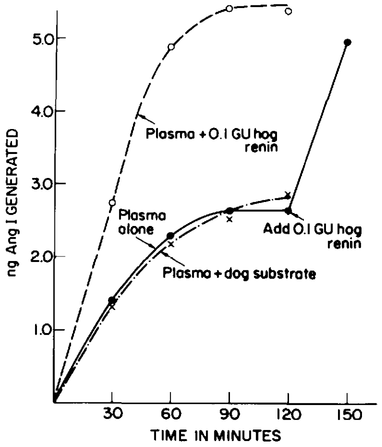

FIG. 1. Time course of generation of angiotensin I in cetacean plasma in the presence and absence of exogenous hog renin or dog substrate.

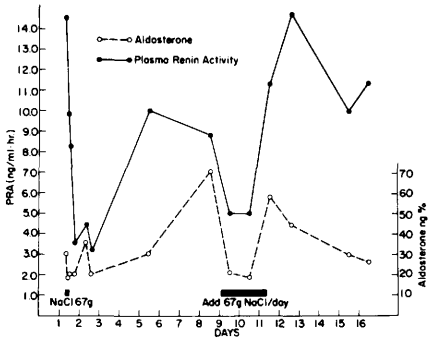

FIG. 2. Variations in PRA and aldosterone concentrations in a dolphin over a 15-day period. Horizontal bars indicate time when the animal was fed. Intervals between bars represent the fasting period.

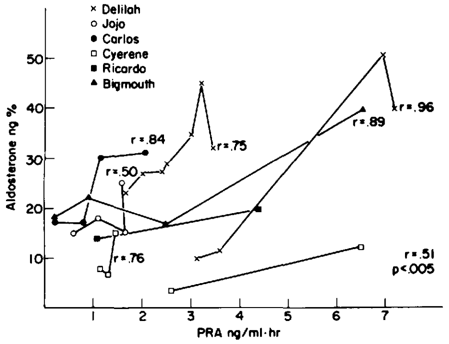

FIG. 3. Relation between PRA and aldosterone levels in six marine mammals. Carlos, Jojo, Ricardo and Bigmouth are sea lions and Delilah and Cyerene are dolphins. Lines connect data points obtained from each animal in one experimental series lasting 16 days. PRA and aldosterone values were varied by feeding NaCl (0.25 g/kg) on days 9–12. In two animals the experiment was repeated 7 months later. In these animals the two sets of data points are represented by two separate lines using the same symbols.

In Fig. 2 are plotted the results for a single animal in which plasma was sampled periodically over a 15-day period, during which the animal was subjected to periodic fasts and periodic treatment with sodium chloride. As can be seen from this figure, the alterations in the levels of aldosterone and PRA follow a similar course. It is clear that both levels fell and rose together over the 15-day period. This animal was treated differently from the animals presented in Fig. 3 in which are plotted the values obtained from all the other animals in which PRA and aldosterone were measured. For most animals there was a significant correlation coefficient between PRA and the concentration of aldosterone of plasma. In addition, the overall correlation coefficient was 0.51 with a *P* value less than 0.005.

Figure 4 shows the results of two experiments on dolphins in which blood and urine were sampled over a 7-hr period while the excretion of sodium in the urine and PRA were also measured. There was an excellent negative correlation between the rate of Na excretion and PRA. No such correlation was obtained when PRA in these same animals was plotted against the simultaneous endogenous creatinine clearance (Fig. 5).

Discussion. The data presented here support the hypothesis that plasma renin activity and aldosterone play a role in the regulation of the salt balance in marine mammals. The highly significant correlation between plasma renin activity and the aldosterone levels of each animal supports the hypothesis that angiotensin is a stimulator of aldosterone secretion in these species. Furthermore, it appears from these data that renin secretion may be regulated by some function of excreted Na, that is, when PRA levels are high, the rate of sodium excretion is low, and vice versa. However, no significant relation was found between creatinine clearance and PRA. Thus it is difficult to assess the nature of the stimulus which causes changes in renin secretion. It should be noted that the wide range of creatinine clearances for each animal is to be expected as the GFR of dolphins is quite labile (7).

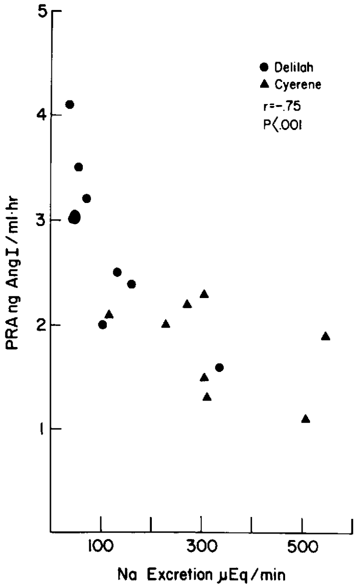

FIG. 4. Relation between the excretion of Na and PRA in two dolphins.

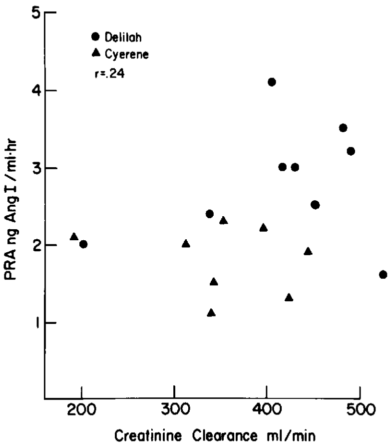

FIG. 5. The lack of correlation between the clearance of creatinine and PRA. Data are from the same animals in Fig. 4.

However, it does appear that the regulation of salt balance by dolphins and pinnipeds may depend in part on an active renin-angiotensin system which stimulates aldosterone secretion. It is possible that aldosterone acts upon renal tubules of these animals in a manner similar to other mammalian species. It remains to the seen, however, what the precise nature of the stimulus is which causes the kidney to alter the secretion rate of renin.

Summary. In both dolphins and sea lions there was found a significant correlation between plasma renin activity (PRA) and the concentration of aldosterone in plasma. Furthermore, an excellent correlation was obtained between the urinary sodium excretion and PRA in two dolphins. These data support the hypothesis that in marine mammals the renin–angiotensin–aldosterone axis plays a role in the regulation of salt balance. However, the signal perceived by the kidney which serves to regulate renin secretion is unknown.

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Received March 29, 1977. P.S.E.B.M. 1978, Vol. 157.