Stimulation by Aldosterone of the Sodium Efflux in Barnacle Muscle Fibres

There is no agreement yet about the mode of action of aldosterone. This is perhaps not surprising especially since most of the information at our disposal has been obtained from studies done on tissues containing asymmetric cells e.g. toad bladder and distal tubules of the kidney. Two theories are prevalent. The first states that aldosterone increases the rate of entry of Na^+ into the cell by inducing the formation of extra permease[1,2]. The second theory states that aldosterone stimulates Na transport by increasing cell metabolism, e.g. by increasing the supply of ATP, or by direct action of the aldosterone-induced protein on the Na pump[3,4]. Because of the difficulties and uncertainties arising from experiments with toad bladder and kidney tubules, and because it is important to know what happens to Na transport in symmetric cells in the presence of aldosterone, attempts were made with the object of seeing whether single giant fibres from crustacea would serve as a satisfactory preparation. This was the approach adopted by BITTAR[5] when he worked with fibres from the crab *Maia squinado*. He found the fibres insensitive to aldosterone but was able to render them sensitive by simply injecting the crab with a dose of the steroid some 15 h before the experiment. The same seems to be true of barnacle fibres. As will be shown in this paper, barnacle fibres acquire sensitivity to aldosterone following exposure of the animal to a dose of the steroid. The obvious implication of this result is that it no longer matters whether one is dealing with a vertebrate or invertebrate but rather whether the pattern of response, following enzyme activation and/or induction, is the same in different tissues.

The experiments were done using muscle fibres from specimens of *Balanus nubilus* and *B. aquila*. These fibres were cannulated and then loaded with ^{22}Na by microinjection as described by BITTAR, CHEN, DANIELSON, HARTMANN and TONG[6]. The artificial sea water used as the bathing medium had the following composition (m*M*): NaCl 465, KCl 10, $CaCl_2$ 10, $MgCl_2$ 10, $NaHCO_3$ 10 and pH 7.8. The method adopted for radiosodium measurement was that described by BITTAR[7] and BITTAR, CALDWELL and LOWE[8]. Aldosterone was obtained from Sigma Chemical Co., St. Louis. All experiments were done between 22 and 24°C.

In the first group of 24 experiments, external application of 10^{-6} *M* aldosterone was without effect on the Na efflux. This led us to explore the possibilty that preexposure of the barnacle in vivo to a dose of aldosterone might render the fibres sensitive to the steroid after a certain period of time. Aldosterone (0.5 mg) was injected into the operculum and the barnacle was left overnight in 1000 cm^3 of artificial sea water (ASW) containing 10^{-6} *M* aldosterone. Fibres isolated from specimens of barnacles injected with this dose of aldosterone and left in ASW for 16 h were found, without exception, to be responsive to externally applied aldosterone As illustrated in Figure 1, external application of 10^{-6} *M* aldosterone caused diphasic stimulation of the Na efflux. The behavior of the Na efflux toward aldosterone, though not always so, was marked by 2 latent periods, the first averaging 36.6 min and the second 96.6 min. Estimates of the magnitude of the 2 stimulatory responses gave mean values of 15.5 and 37.3% ($n = 24$). It is instructive to give an interpretation of the kinetics of the observed diphasic response, since the first phase is characterized by a slow step-up in the rate of Na afflux followed by a reduction in its rate of decline. Furthermore, inspection of the rate coefficient plot reveals that the rate coefficient slowly became a constant during this first phase of aldosterone action. Kinetic results of this type could be taken to mean that aldosterone acts by mobilizing the fraction of sequestered sodium (see BITTAR[9]).

The sensitivity of the fibres to aldosterone varied rather widely. As shown in Figure 2a, external application of 10^{-5} *M* aldosterone caused monophasic rather than diphasic stimulation, which was delayed and brief. As expected, however, it also caused a prompt reduction in the rate of decline of the Na efflux. That indeed the fractional loss of Na* had become a constant is shown in Figure 2b, which is the rate coefficient plot. Evidence of a mere change in slope in the efflux plot, and of constancy of the fraction of Na* lost per second also comes from experiments done with 10^{-10} *M* aldosterone, a concentration which falls within the physiological range.

Lastly, it is not possible to overlook the fact that stimulation by aldosterone of the Na efflux in barnacle fibers is a result which is the opposite of that found by BITTAR[5] in *Maia* fibres. To account for slowing down of Na transport in *Maia* fibres one would have to suppose

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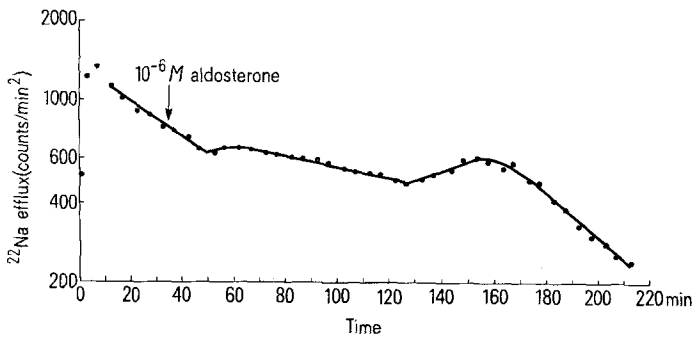

Fig. 1. Semilog plot showing Na efflux before and after external application of 10^{-6}*M* aldosterone.

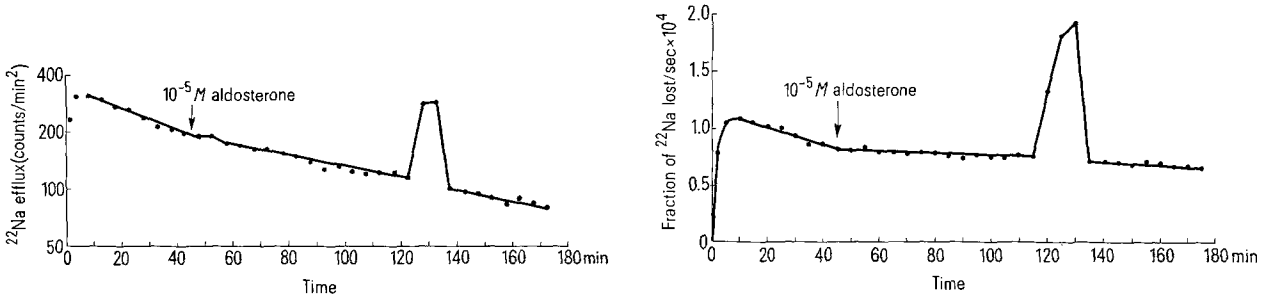

Fig. 2. Effect of external application of $10^{-5}M$ aldosterone. a) Efflux plot. b) Rate coefficient plot.

that aldosterone increased the size of the internally sequestered fraction of Na or increased the Na influx. Whatever one may think of these contradictory observations, it looks very much as if we now have the best of reasons for intensifying our work with aldosterone[10].

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Zusammenfassung. Es wurde gezeigt, dass die Natrium-Ausscheidung in der Muskelfaser von Entenmuskeln auf Aldosteron reagiert.

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Neurotoxic Effect of Leptophos

While organophosphorus insecticides may produce acute poisoning, with temporary muscle weakness from its cholinergic activity, only a few of these compounds produce delayed locomotor ataxia, which first develops 7 days or more after the administration of the compound, as described by Smith and Elvove[1], Smith and Lillie[2] and Johnson[3]. This effect has been demonstrated in 12 species including rodents, mammals and birds by Aldridge et al.[4]. In the original reports, the clinical condition in hens was very similar to that in man (Cavanagh[5]).

In the present study, 3 insecticides used currently in Egypt were tested for any delayed meurotoxicity in chickens. 2 of these compounds, leptophos (Phosvel) and cyolane were accused of poisoning and killing about 1,300 water buffaloes in the Nile delta in Summer, 1971 (Near East News Roundup)[6].

Materials and methods. Male chickens of local breed (Alexandrian) each weighing approximately 2 kg, and 5 months old, were placed in open pens. Each bird received a single dose of the insecticide in a gelatin capsule in the case of cyolane (2-(diethoxyphosphenyl)-1, 3-dithiolane) (97%) and cytrolane (2-(diethoxyphosphenyl)-4-methyl-1, 3-dithiolane) (98%, both obtained from American Cyanamide Co., Princeton, N.J. USA). However, with leptophos (*O*-(4-bromo-2, 5-dichlorophenyl) *O*-methyl phenylphosphonothioate) it was necessary to dissolve it in corn oil and administer it with oral intubation, since large doses were required to reach the toxic level. The birds were given free access to food and water. For each dose at least 5 birds (5–9 birds) were used. The dose killing 50% of the birds within 24 h is reported as LD_{50}. The surviving birds were observed every 2 days for 60 days or until they died. An adequate untreated group of chickens was included in every test.

Results and discussion. The present data demonstrate that cyolane and cytrolane have very high acute toxicity to male chickens. The LD_{50} for a single oral dose is 5.2 and 2.8 mg/kg for cyolane and cytrolane. The 2 values were used to determine the delayed neurotoxic effect for these 2 compounds. A single dose of 5.2 and 2.8 mg/kg of cyolane and cytrolane, respectively, were administered to 10 birds for each compound, and the surviving birds were observed for 2 months. No delayed neurotoxic symptons were detected in any of the surviving birds.

Our results show that the acute toxicity in male chickens to leptophos is very low. The LD_{50} for a single oral dose is 4,700 mg/kg. The Table shows that the lowest dose tested, i.e., 140 and 160 mg/kg, caused no neurotoxicity. However, higher doses (180–3,000 mg/kg) caused delayed neurotoxic effect in some cases. This condition was characterized by ataxia developing 8 to 13 days after the beginning of the experiment. Early signs were less activity, loss of appetite, loss of feathers, lowering of hindquarters and reluctance to stand. As time passed, the signs progressed as incoordination of the leg movement, legs sprawling out in front, inability to stretch the legs, tendency to lay down on the side and paralysis of the wings. Once weakness and ataxia had appeared in the birds, decline was rapid, paralysis occurred and bird might collapse and die with respiratory failure. Recovery was never observed in any bird having developed ataxia. The severity of the neurotoxic signs and the number of birds that developed the condition were dose-dependent; but not matter how great the dose, the latent period before a neurotoxic appeared was never less than 8 days.

In conclusion, the present results clearly demonstrate that leptophos causes delayed neurotoxic effects when administered orally to male chickens. Since it is assumed

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