J. exp. Biol. **114**, 355–364 (1985)

TEMPERATURE ACCLIMATION OF INTESTINAL Na TRANSPORT IN THE CARP (*CYPRINUS CARPIO* L.)

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Accepted 2 August 1984

SUMMARY

1. Carp intestine mounted *in vitro* has a positive serosal potential and a net Na absorption greater than the short-circuit current.

2. At 30°C *in vitro*, tissues from 10°C-acclimated fish are thought to show heat-damage.

3. When measured at 10°C *in vitro*, intestine from fish acclimated to 10°C shows a greater rate of sodium transport than that from 30°C-acclimated fish.

4. Mucosal application of amphotericin B, at 10°C *in vitro*, increases short-circuit current and net Na flux in both 10°C- and 30°C-acclimated fish but does not diminish the difference in Na transport between the two groups, under conditions when the apical membrane permeability is not limiting.

5. It is concluded that the principal acclimatization in carp intestine to low temperature is *via* an increased basolateral membrane Na pumping capacity.

INTRODUCTION

Freshwater teleosts have often been used for the study of acclimatization to changes in environmental temperature (e.g. Prosser, 1973; Hazel & Prosser, 1974). Modification of intestinal transport function is an important part of the overall compensatory response to seasonal temperature changes and has been extensively investigated (Smith, 1970, 1976; Groot, Albus, Bakker & Dekker, 1983). Adaptive changes in salt transport in this epithelium may involve regulation at one or more of three sites; the apical membrane entry step, basolateral membrane Na pumping activity and the junctional permeability (see Fig. 4). A useful comparison in this context may be regulation of intestinal NaCl transport during salt adaptation in euryhaline teleosts (e.g. Ellory & Gibson, 1983). Previous studies using the everted-sac technique or measuring glucose-evoked potentials cannot be interpreted easily in terms of these regulatory sites (see Discussion).

Key words: Acclimation, intestine, epithelium.

In the present experiments we have measured bidirectional Na fluxes under voltage-clamp conditions in a stripped intestinal preparation from carp acclimated to 10°C or 30°C for long periods. We have used mucosal application of the polyene antibiotic amphotericin B to increase the permeability of the apical membrane to univalent ions. This has enabled us to assess separately the contributions of transport processes in the apical and basolateral membranes of the intestinal epithelial cells to the overall adaptation.

Our results suggest that regulation of intestinal Na transport is achieved mainly by changes in the functioning of basolateral transport processes, rather than *via* the apical membrane.

A preliminary account of this work has been published (Cossins, Ellory & Gibson, 1984).

METHODS

Animals

Carp (*Cyprinus carpio*) weighing 0·25 to 0·5 kg were acclimated in 500-l aquaria at environmental temperatures of 10, 20 or 30°C (all ±1°C) for at least 12 months. Fish at 30°C were fed twice daily, at 20°C and 10°C once daily, with mealworms, or occasionally with trout pellets. Photoperiod was maintained at 16 h light, 8 h dark.

Experimental procedure

Animals were stunned by a blow to the head and then killed by double-pithing. The abdomen was opened and the entire intestine removed. Strips of intestine, immediately posterior to the entry of the bile-duct, were washed with saline, then opened longitudinally and pinned mucosal-side down whilst the serosal muscle layer was peeled off. This removal was easier in 20°C and 30°C fish than in 10°C fish, but it was interesting that the 30°C mucosa seemed most delicate. The pieces of stripped intestine were stretched carefully and mounted in a modified Ussing chamber with an exposed area of 1·2 cm^2 and a half-chamber volume of 2·6 ml. Chamber temperature was maintained at the required *in vitro* values (±1°C) by circulating fluid through a water-jacket. Usually two pieces of intestine from each of two fish were studied simultaneously in a set of four chambers; one piece was maintained at 10°C, the other at 30°C. The experimental saline had the following composition, in mmol l^{-1}: NaCl, 130; KCH_3COOH, 10; $NaHCO_3$, 25; $MgSO_4$, 1·1; $CaCl_2$, 2·5; alanine, 2; and glucose, 10; and was bubbled continuously with gas, 95 % O_2/5 % CO_2 giving a pH of 7·2 at 20°C.

Electrical measurements

Electrical parameters were measured using standard techniques established in the measurement of small potential differences (PDs) (less than 0·5 mV) see Ramos & Ellory, 1981. Transepithelial PD (expressed in mV with the polarity of the serosal relative to the mucosal side) was measured with a pair of calomel electrodes connected to the Ussing chamber *via* 3 % agar-saline bridges, each ending about 2 mm from the tissue. Chambers were allowed to equilibrate for at least 90 min

before mounting the tissue and dismantled if the PD between members of an electrode pair was >0·1 mV or if drift was >0·05 mV h^{-1} – to ensure stability. A conventional voltage-clamp was used to inject an external current (I_{sc}, expressed in $\mu A\,cm^{-2}$, with a current passed from serosa to mucosa taken as positive) *via* a pair of Ag/AgCl electrodes and agar-saline bridges to short-circuit the epithelium continuously, except for a few seconds twice every 20 min to record the PD. Compensation for the solution resistance between the voltage electrodes was carried out during the equilibration period by current injection and measurement of the resulting PD across the electrodes (done automatically by the voltage-clamp apparatus) and an offset was then introduced into the clamp circuit to allow for this resistance under voltage-clamp conditions. The circuit diagram is given by Ramos (1980). Transepithelial resistance (Ωcm^2) is taken as $(PD/I_{sc})\times 10^3$. Short-circuit current rapidly attained a stable value which was maintained for long periods, except for 10°C tissues at high temperature.

Na flux measurements

Bidirectional Na fluxes were measured simultaneously using ^{24}Na on the mucosal side (1–2 $\mu Ci\,ml^{-1}$) and ^{22}Na on the serosal side (0·1–0·2 $\mu Ci\,ml^{-1}$) to give the net Na flux. Isotope equilibration was allowed to take place for at least 1 h before samples were taken. Radioactive solutions were subsequently replaced every 20 min. ^{24}Na samples (^{24}Na transported to the serosal side, J_{ms}) were counted immediately by Cerenkov radiation in a Packard 5880 Scintillation counter and again after the decay of ^{24}Na to correct for the ^{22}Na contribution. Following ^{24}Na decay, ^{22}Na samples (^{22}Na transported to the mucosal side, J_{sm}) were added to 6 ml of scintillation fluid (Pico-Fluor^{TM}30) and were counted in a Packard Tricarb scintillation spectrometer. Alternatively, ^{22}Na was counted directly in a Packard Autogamma scintillation spectrometer.

^{22}Na was obtained from Amersham International (Buckinghamshire, England) as $^{22}NaCl$ in an aqueous carrier-free solution (200 $\mu Ci\,ml^{-1}$); ^{24}Na was made by the Universities Research Reactor (Warmington, England) as $^{24}Na_2CO_3$ which was neutralized with excess HCl, evaporated to dryness and dissolved in a small volume of distilled water (at 2 mCi ml^{-1}).

Amphotericin

Amphotericin was obtained commercially as Fungizone (50 mg amphotericin; 41 mg sodium desoxycholate buffered with 20·2 mg sodium phosphates) and was used as a saturated suspension at 80 $\mu g\,ml^{-1}$.

Abbreviations

Apart from those defined above: J_{ms}, mucosa-to-serosa Na flux; J_{sm}, serosa-to-mucosa Na flux; J_{net}, net Na flux (absorption positive) ($=J_{ms}-J_{sm}$). Fluxes are expressed in $\mu mol\,cm^{-2}\,h^{-1}$.

Statistics

Results are expressed as mean ± S.E.M. of (N) experiments. Comparisons were made using unpaired Student's t-test.

RESULTS

The overall acclimation response

Electrical parameters and Na fluxes

Short-circuit current was measured in stripped carp intestine at three *in vitro* temperatures, for tissues derived from animals acclimated to either room temperature or hot (30°C) or cold (10°C) environments (see Fig. 1A,B and Table 1). The results presented initially in Fig. 1A represent I_{sc} measured *in vitro* at the *in vivo* acclimation temperature and show how the tissue might be expected to function *in vivo*. The increase in I_{sc} from 10°C to 30°C is not linear but may follow an exponential relationship.

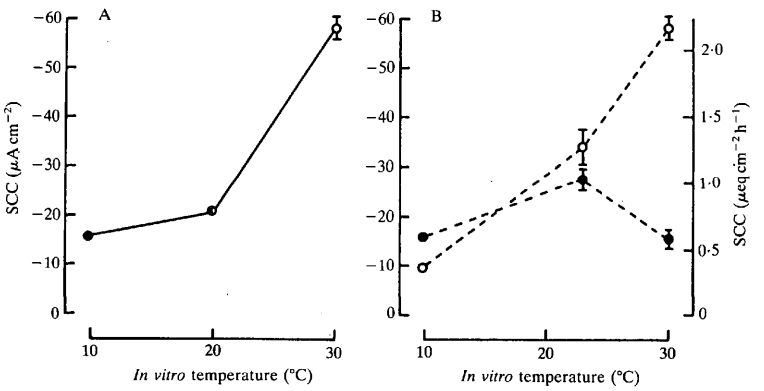

Fig. 1. (A) Isothermal response: short-circuit current (SCC) *vs* temperature. ● Carp acclimated to 10°C, $N = 21$; ◐ Carp acclimated to 20°C, $N = 24$; ○ Carp acclimated to 30°C, $N = 9$. Data represent mean ± S.E.M. (N). (B) Short-circuit current *vs in vitro* temperature for carp acclimated to 10°C or 30°C. At 10°C $N = 21$ for 10°C fish and $N = 19$ for 30°C fish; at 20°C, $N = 6$ and 4 respectively; at 30°C, $N = 9$ for both groups.

At 10°C *in vitro*, tissues from fish acclimated to that temperature show significantly greater transport rates than those from 30°C fish (Fig. 1B, Table 1), both as regards I_{sc} [$15{\cdot}7 \pm 1{\cdot}6$ (21) *vs* $9{\cdot}5 \pm 1{\cdot}2$ (19) $\mu A\,cm^{-2}$, $P < 0{\cdot}01$] and net Na absorption [$2{\cdot}52 \pm 0{\cdot}29$ (20) *vs* $1{\cdot}48 \pm 0{\cdot}16$ (20) $\mu mol\,cm^{-2}\,h^{-1}$, $P < 0{\cdot}01$]. Similarly, transport rates at 30°C are greater in fish acclimated to that temperature, $4{\cdot}61 \pm 0{\cdot}49$ (9) $\mu mol\,cm^{-2}\,h^{-1}$ for 30°C-acclimated fish and $2{\cdot}20 \pm 0{\cdot}40$ (8) for 10°C fish. Indeed, at this high temperature, 10°C tissues are probably showing heat-damage, where the I_{sc} is initially high but falls rapidly to the low, apparently stable, value recorded during the flux periods. In all other cases at 10°C and 30°C *in vitro*, tissues rapidly attain a stable value of I_{sc} and such lability is absent (see

Table 1. *A comparison of electrical parameters and Na fluxes measured at 10°C* in vitro *in carp acclimated to 10°C and 30°C*

	Acclimation temperature	
	10°C	30°C
I_{sc} (μA cm^{-2})	−15·7 ± 1·6 (21)	−9·5 ± 1·2 (19)
PD (mV)	+1·5 ± 0·1 (21)	+0·9 ± 0·1 (19)
R (Ωcm^2)	110 ± 17 (21)	118 ± 16 (19)
J^{Na}_{ms} (μmol cm^{-2} h^{-1})	6·36 ± 0·53 (20)	5·45 ± 0·60 (20)
J^{Na}_{sm}	3·84 ± 0·40 (20)	3·97 ± 0·56 (20)
J^{Na}_{net}	2·52 ± 0·29 (20)	1·48 ± 0·16 (20)

Values are mean ± S.E.M. (*N*).

Table 2. *Stability of I_{sc} (μA cm^{-2}) with time after mounting at 10°C and 30°C* in vitro

In vitro temperature	I_{sc} after 30 min	I_{sc} during fluxes (90–150 min)
A 10°C-acclimated carp		
10°C	16·1 ± 1·9 (21)	15·7 ± 1·6 (21)
30°C	45·0 ± 5·7 (9)	15·6 ± 3·5 (9)
B 30°C-acclimated carp		
10°C	10·5 ± 1·1 (19)	9·5 ± 1·2 (19)
30°C	51·7 ± 4·7 (9)	58·2 ± 4·8 (9)

Values are mean ± S.E.M. (*N*).

Table 2). This view is substantiated by the inability of 10°C fish to survive at 30°C for any length of time.

A further important observation in Table 1 is the close similarity of tissue resistance and J_{sm} (passive backflux of Na) between the 10°C and 30°C fish measured at 10°C. This implies that the functional transport area, per unit serosal area of stripped intestine, is similar in the two groups, in contrast with previous work of Groot *et al.* (1983) in goldfish intestine.

If the net Na absorption (Table 1) is compared with the I_{sc} (Fig. 1B), in every case the net Na transport is much greater than can be accounted for by the current, expressed as an equivalent flux of univalent cations. Thus for both 10°C or 30°C fish at 10°C the net Na flux is about four-fold greater than the current, whilst at 30°C this ratio is about two-fold for the 30°C fish.

Na transport at 10°C

The subsequent experiments were all conducted at 10°C in an attempt to establish the mechanism which accounts for the greater Na flux in the 10°C-acclimated fish at this temperature as compared with the 30°C-acclimated fish.

In both groups of tissues, mucosal application of amphotericin B (80 μg ml^{-1}) caused an immediate stimulation of current (Fig. 2) with a subsequent increase in

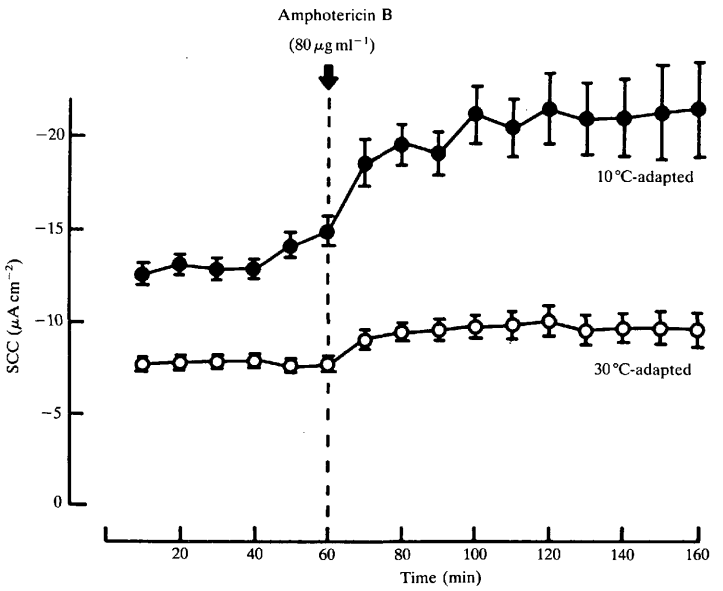

Fig. 2. Effect of amphotericin B on the short-circuit current (SCC) in carp intestine at 10°C *in vitro*. ● 10°C-acclimated carp, $N = 11$. ○ 30°C-acclimated carp, $N = 12$. Data represent mean ±S.E.M. (N).

net Na transport (Fig. 3). Any diffusion potentials across the cell membranes will be immediately affected by the application of amphotericin and could produce a rapid change in I_{sc}, whilst the slower response of the fluxes could be caused partly by the time required for new equilibration of the intracellular Na pools with the isotope tracers, following perturbation. The difference in net Na absorption between the two groups was not diminished by amphotericin; after treatment the net Na flux was $2{\cdot}71 \pm 0{\cdot}23$ (41) μmol cm^{-2} h^{-1} for 10°C fish and $1{\cdot}84 \pm 0{\cdot}16$ (46) for 30°C fish compared with control values of $1{\cdot}98 \pm 0{\cdot}15$ (33) and $1{\cdot}42 \pm 0{\cdot}13$ (34) respectively, and shows a tendency to increase. It is concluded, therefore, that the Na pumping capacity of the 30°C fish was less than that of the 10°C fish under conditions where the mucosal membrane permeability is not limiting.

DISCUSSION

When the rate of a physiological process is measured as a function of temperature it often gives a bell-shaped curve, whose position alters with the environmental temperature to which the animal is acclimated. The present short-circuit current

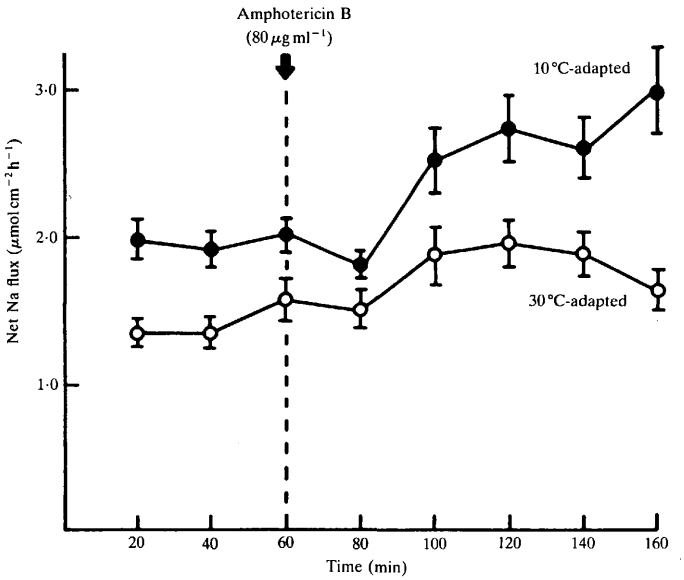

Fig. 3. Net Na flux *vs* time, in carp intestine at 10°C *in vitro*. ● 10°C fish, $N = 11$. ○ 30°C fish, $N = 12$.

data for carp (Fig. 1B) reflect this classical pattern. Thus the curve for 10°C-acclimated fish is shifted to the left and has a decreased maximum compared to that for 30°C fish, although the downward limb for the latter, at high temperature, was beyond the experimental temperature range used in this study (e.g. Precht, Christophersen, Hensel & Larcher, 1974).

The results for Na absorption as a function of temperature (Table 1 and text) are not as complete as those for I_{sc} but show a similar trend. However, net Na transport is considerably greater than I_{sc} in this preparation and therefore must be accompanied by movement of other unidentified cations or anions. In many tissues this discrepancy is the result of Na^+/H^+ exchange (sometimes linked to Cl^-/HCO_3^- exchange) at the apical membrane. Na^+ entry *via* a Na^+/H^+ antiport would have the additional effect of raising the body fluid pH. At low environmental temperatures one response of the fish is to elevate plasma HCO_3^- concentration and hence pH (e.g. Reeves, 1977; White & Somero, 1982) and it is perhaps significant in this respect that the ratio between net Na absorption and I_{sc} in Fig. 1B and Table 1 is larger at low temperature.

At 10°C *in vitro*, tissues from fish acclimated to that temperature show a greater

rate of net Na transport than those from 30°C fish. Initially, we must eliminate a purely morphological cause. Groot *et al.* (1983) observed an increase in mucosal folding in intestine from 10°C-acclimated goldfish compared with 30°C fish. The greater transporting ability of the 10°C tissues may, therefore, simply reflect an increase in the effective transport area rather than a change in cell function. However, we discount this possibility in our preparation because of the similarity of transepithelial resistance and J_{sm}, at 10°C *in vitro*, (Table 1) between the two groups of fish. For the increase in net Na absorption to be caused morphologically by an increase in mucosal cell number *per unit serosal area*, one would expect a concomitant decrease in transepithelial resistance sufficiently large to be observed in our measurements. This difference from the findings of Groot *et al.* (1983) may be a result of stripping off the serosal muscle layer and stretching the intestine on mounting *in vitro* in the present experiments. Also it is not a simple procedure to relate functional transport area and mucosal area and an increase in the latter may not increase the actual transport area.

In terms of the functional adaptation of epithelial Na transport, conventional models would suggest three possible regulatory sites (as outlined above): (1) apical Na permeability, (2) basolateral Na pumping and (3) junctional permeability (see Fig. 4). The latter can be discounted since changes in junctional permeability would be expected to alter transepithelial resistance and J_{sm} and, as mentioned above, these are very similar in the two groups of fish.

We have attempted to assess separately the contributions of apical permeability and basolateral transport using amphotericin B, mucosal application of which bypasses the apical entry step by increasing the permeability to univalent ions (e.g.

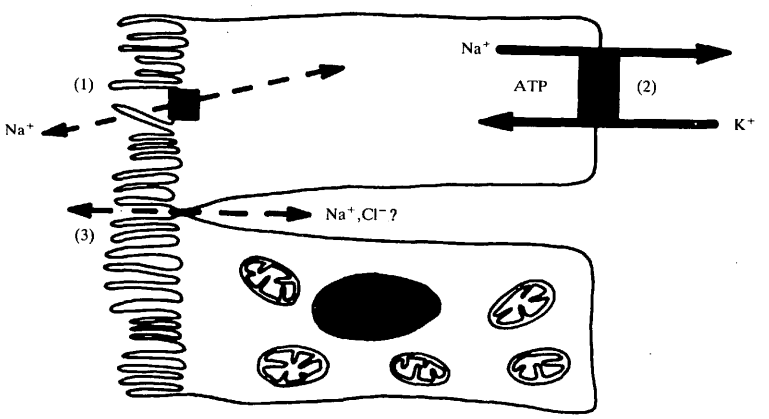

Fig. 4. Schematic diagram of transepithelial Na transport illustrating the possible regulatory sites. (1) Apical membrane Na permeability; (2) basolateral membrane pump activity; (3) junctional permeability.

Rose & Nahrwold, 1976; Ellory, Lau & Gibson, 1984). Intracellular Na concentration will rise and stimulate Na transport if intracellular concentration was previously limiting. Under these conditions, one would expect net Na flux to become more similar in the two preparations if there is no acclimation in basolateral membrane function. In fact the converse occurs (Fig. 3) indicating a greater capacity for basolateral membrane Na transport in the cold-adapted fish. We cannot identify further the cause of the observed acclimation in pump activity – it could result from changes in turnover rate, membrane fluidity or ATP supply (e.g. Smith & Ellory, 1971; Cossins, 1983; Wodtke, 1981).

Whilst the effects of amphotericin imply that acclimation in the cold-acclimated intestine can be mediated by changes in the basolateral Na pump activity, they cannot be taken as unequivocal evidence that there is no acclimation in apical membrane permeability. However, they do show that such a change is unnecessary. Further, the small stimulation of net Na flux by amphotericin in the hot-adapted tissues indicates little reserve capacity for Na transport following an increase in apical Na permeability. One could argue that Na permeability is already sufficiently high in the control situation to saturate the basolateral Na pumping system.

The present results using amphotericin cannot be compared directly with other results using everted-sac preparations or measuring glucose-evoked potentials (e.g. Smith, 1976; Groot *et al.* 1983), which are difficult to interpret in terms of apical and basolateral membrane acclimations, because an effect at one site could well affect the functioning of the other. For example, changes in glucose-evoked potentials following temperature acclimation are taken to reflect a change in the apical membrane permeability, but will also be dependent on the Na pump. Whilst it is obvious that changes in apical membrane Na permeability can affect the basolateral membrane Na pump activity by limiting its substrate, the converse may also occur, and there is evidence for a negative feedback mechanism in which pump activity determines apical Na permeability (e.g. Taylor & Windhager, 1979). Although our approach in this paper has been an attempt to define the acclimation response in terms of separate contributions of apical and basolateral membranes, *in vivo* both have an important role, and integration of function between the two cannot be neglected when considering the mechanism of acclimation.

The apparent acclimation of pump activity shown here agrees surprisingly well with the data of Smith & Ellory (1971) of Na/K-ATPase activity measured in homogenates from goldfish intestine following long-term temperature acclimation. These authors showed a 40 % lower specific activity in 30°C fish compared with 16°C fish; our data (comparing over a larger temperature range) show that 30°C fish at 10°C have a net Na flux about 41 % smaller than 10°C-acclimated fish. Additional results of Smith & Ellory suggest that changes in Na/K-ATPase activity may not be involved in acclimation of Na transport *during* the actual acclimation process because the time-course of the two differ. However, the activity of homogenates does not always bear a constant relationship to functional activity *in vivo* and this is especially so in non-steady-state conditions. The amphotericin technique is a useful method for substantiating their interpretation.

J. S. Gibson thanks the MRC for financial support.

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