

THE EFFECTS OF OSMOREGULATORY SOLUTES ON TENSION GENERATION BY DOGFISH SKINNED MUSCLE FIBRES

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(Received 13 July 1981)

Many marine organisms accumulate high concentrations of solutes in their tissues to maintain osmotic balance. Osmoregulatory solutes are usually end-products of metabolism rather than inorganic ions (Prosser, 1973). For example, skeletal muscle from marine elasmobranchs, holocephalans and the coelacanth contains high concentrations of both urea (300-600 mM) and methylamine compounds, such as trimethylamine oxide (175-250 mM) (Pang, Griffith & Atz, 1977). Urea is a potent protein destabilizer, and most elasmobranch enzymes examined are inhibited by urea (Yancey, 1978). However, it has recently been found that TMAO acts as a general protein stabilizer and can offset the effects of urea on enzyme K_m and maximal velocity *in vitro* (Yancey & Somero, 1979, 1980).

Skinned fibres provide a more complex and more physiological model than isolated proteins, and in the present study the effects of osmoregulatory solutes on tension generation in skinned fibres isolated from dogfish myotomal muscle have been investigated.

Dogfish, *Scyliorhinus canicula*, 55-65 cm long, were obtained from the Millport Marine Laboratory, Isle of Cumbrae, Scotland, and were kept in filtered, recirculated sea water at 10 ± 1 °C. Fish were killed by stunning and pithing. Small strips of fast and slow myotomal muscle were excised from the larger myotomes near the midline and below the dorsal fin, pinned to cork boards and stored in ice-cold Ringer (Meiss, Jenson & Prosser, 1974). Bundles of 10-20 fibres were freed at their myoseptal ends, teased from the main muscle mass, and immersed in a glass trough 2 mm deep containing silicone fluid at 0-5 °C. A small drop of standard relaxing solution (R) was injected around each bundle. R contained 10 mM imidazole-HCl (pH 7.0 at 0.5 or 8 °C), 110 mM-KCl, 5 mM EGTA, 3 mM-MgCl₂, 2.5 mM ATP, 10 mM phosphocreatine, and 20 u. ml⁻¹ creatine kinase. Maximally activating solution (A) was made by the addition of 4 mM-CaCl₂ (pCa 5.52). Activating and relaxing solutions were also made containing 330 mM urea or 180 mM TMAO or both, the intracellular concentrations found in another dogfish species (Robertson, 1975). Single fast fibre segments or bundles of slow fibres, 50-200 µm in diameter and 1-2 mm long, were attached to the apparatus as previously described (Altringham & Johnston, 1981),

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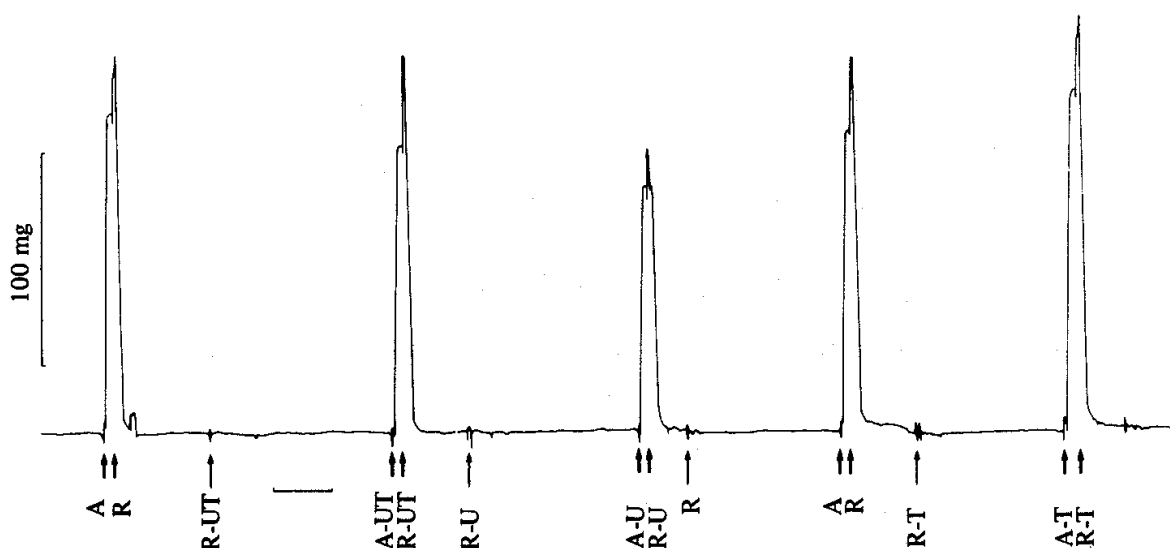


Fig. 1. The effect of osmoregulatory solutes on P_0 . Results from a typical slow fibre preparation at 0.5°C . Time calibration = 2 min during contraction cycles, 4 min during incubations. Arrows indicate solution changes. A = activating solution; R = relaxing solution; U = +urea; T = +TMAO; UT = +urea and TMAO.

Table 1. *The effect of physiological concentrations of osmoregulatory solutes on maximum isometric tension*

Fibre type (no. of fibres)	Temperature ($^\circ\text{C}$)	$(P_0(\text{solute})/P_0) \times 100\%$		
		Urea	TMAO (no. of observations)	Urea + TMAO
Fast ($n = 4$)	0.5	68.5 ± 2.29 ($n = 6$)	106.5 ± 2.51 ($n = 4$)	90.4 ± 0.95 ($n = 6$)
Slow ($n = 3$)	0.5	$64.0 \pm 2.51^*$ ($n = 3$)	108.7 ± 2.85 ($n = 3$)	89.5 ± 1.50 ($n = 3$)
Slow ($n = 6$)	8	$77.6 \pm 1.73^*$ ($n = 9$)	104.0 ± 1.29 ($n = 4$)	95.1 ± 1.43 ($n = 9$)

* $P < 0.01$.

and sarcomere length, measured by laser diffraction, was set to $2.3 \mu\text{m}$. Fibres were skinned by a 30 min soak in R containing 1% Brij 58 (Sigma Chemicals), before being transferred to R for 10 min. Maximal isometric tension (P_0) was initially measured in each fibre by immersion in A. Each preparation was then incubated for 10 min in one of the urea/TMAO relaxing solutions before activation in the presence of the same solute(s): 5–10 activation–relaxation cycles were performed on each preparation, with ‘standard’ cycles at regular intervals to monitor any decrease in P_0 with time.

The experimental protocol is illustrated in Fig. 1. Individual fibres could be taken through up to 15 standard activation/relaxation cycles with a 10% decrease in P_0 . Physiological concentrations of urea caused a large decrease in P_0 (Table 1). The depression in P_0 at 0.5°C was greater than at 8°C . A similar temperature dependence of urea inhibition on the enzyme kinetics of isolated proteins has been noted by Rajagopalan, Fridovich & Handler (1961). In all experiments, TMAO produced a

small increase in P_0 . More importantly, in the presence of TMAO, the depression of P_0 by urea was almost fully reversed. Similar results were obtained with both fibre types (Table 1).

TMAO and other methylamines appear to be general protein stabilizers in that urea inhibition can be reversed in both elasmobranch and non-elasmobranch enzymes systems (Yancey & Somero, 1979, 1980). For example, urea decreases the thermal stability of bovine ribonuclease and increases the apparent Michaelis constant (K_m) of dogfish skeletal muscle creatine kinase for ADP (Yancey & Somero, 1979, 1980). These effects can be largely offset by the inclusion of trimethylamine oxide in the assay medium at concentrations around half that of urea (Yancey & Somero, 1979, 1980). The mechanism behind the effects of osmoregulatory solutes on tension generation (Table 1) and enzyme kinetic properties is unknown (for review see von Hippel & Schleich, 1969).

The small inhibitory effect on P_0 still present may well be accounted for by the absence of other osmoregulatory solutes (e.g. betaine and sarcosine) from the activating/relaxing solutions.

Thus it would appear that muscle contraction in elasmobranchs is not dependent on unique urea-adapted proteins, but on the accumulation of activating and inhibiting osmotic solutes at counteracting ratios.

Supported by a grant from the Science and Engineering Research Council.

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