The effect of temperature on the ouabain-sensitivity of Na^+-K^+-activated ATPase and fluid secretion by the Malpighian tubules of *Locusta*

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Summary. Temperature markedly affected the ouabain-sensitivity of both the Na^+-K^+-activated ATPase activity and the secretion of fluid by the Malpighian tubules of *Locusta*. Varying the K^+ concentration in the bathing medium did not affect the ouabain-sensitivity of the fluid secretory process.

Previous studies have revealed the presence of a Na^+-K^+-activated ATPase in microsomal preparations from the Malpighian tubules and hindgut of *Locusta*[1,2]. This enzyme has been implicated in cation and fluid secretion across the Malpighian tubule wall by virtue of the fact that these processes are inhibited by the cardiac glycoside, ouabain[1,3]. However, the literature concerning the ouabain-sensitivity of insect epithelia is conflicting (see review by Anstee and Bowler[4]). For example, in contrast to the results referred to above[1,3], other workers[5] have failed to demonstrate that fluid secretion by *Locusta* Malpighian tubules is inhibited by ouabain. It seems likely that the explanation for such differences is due to variations in experimental procedures; in particular the temperatures at which determinations were made and the composition of the bathing media.

Ouabain-inhibition of Na^+-K^+-activated ATPase has been reported to be extremely temperature sensitive[6]. Similarly, high K^+ concentrations have been shown to antagonize ouabain-inhibition of Na^+-K^+-activated ATPase[7-10]. The present study has been carried out to determine the extent to which temperature affects the ouabain-sensitivity of both the Na^+-K^+-activated ATPase from *Locusta* Malpighian tubules and the in vitro secretion of fluid by such tubules. In addition, the effect of various concentrations of K^+, on the ouabain-sensitivity of tubule fluid secretion, has been studied.

Material and methods. Mature adult locusts, *Locusta migratoria* L. were used and these were taken from populations maintained under crowded conditions at 28 ± 0.5 °C and 60% relative humidity. In vitro measurements of fluid secretion by the Malpighian tubules and the preparation of a membrane microsomal fraction were carried out as described previously[1]. All measurements were made at 30 ± 0.1 °C unless otherwise stated. The composition of the 'normal' Ringer's solution was: 100 mM NaCl, 8.6 mM KCl, 2 mM $CaCl_2$, 4 mM NaH_2PO_4, 4 mM $NaHCO_3$, 8.5 mM $MgCl_2$, 34 mM glucose, 25 mM HEPES (N.2-hydroxyl ethyl piperazine N'.2-ethanesulfonic acid), 11 mM NaOH (pH 7.2).

To determine the effect of temperature on the ouabain-sensitivity of fluid secretion. The rate of secretion by in vitro preparations was determined by measuring the diameter of the secreted droplets of 'urine' at 5 min intervals over a period of 35 min in 'normal' Ringer's solution. At the end of this time, the 'normal' Ringer's solution was replaced by a fresh solution which had either the same composition (the control) or 1 mM ouabain added. The rate of secretion was redetermined, after an equilibration period of 30 min, for a further 35 min. Determinations were carried out at 15, 20 and 30 °C.

To determine the effect of K^+ concentration on the ouabain-sensitivity of fluid secretion: The rate of fluid secretion by in vitro preparations was determined in Ringer's solution in which the K^+ concentration was either 10, 20 or 40 mM. The Na^+ concentration of the solution was adjusted to maintain the same cation concentration as 'normal' Ringer's solution; the composition of the experimental solutions being otherwise identical to that of 'normal' Ringer's solution. After the initial rate of secretion had been determined at 5 min intervals over a period of 35 min, the tubules were surrounded by either the same experimental solution (the control) or the same experimental solution containing 1 mM ouabain. Once again the rate of secretion was redetermined for 35 min following an equilibration period of 30 min.

To determine the effect of temperature on the ouabain-sensitivity of Na^+-K^+-activated ATPase. Appropriate ionic media (1.5 ml) were thermoequilibrated at 15, 20 and 30 °C for 15 min in boiling tubes and the reaction started by adding 0.5 ml of microsomal suspension. The reaction was stopped by adding 4 ml of a 1:1 mixture of 1% cirrasol ALN-WF and 1% ammonium molybdate in 0.9 M sulphuric acid. Enzyme activity was measured by determining the amount of inorganic phosphate released. Following centrifugation to remove any protein that had precipitated, the tubes were allowed to stand at room temperature for exactly 10 min. The intensity of the yellow colour which developed during this time was read at 390 nm and was proportional to the amount of inorganic phosphate present. 3 reaction media were used: 1. 4 mM $MgCl_2$; 2. 4 mM $MgCl_2$, 100 mM NaCl and 20 mM KCl; 3. 4 mM $MgCl_2$, 100 mM NaCl, 20 mM KCl and 1 µM ouabain. Each medium contained 3 mM ATP (Tris salt) and was made up in 50 mM histidine - HCl, pH 7.2. Protein determinations were made by the method of Lowry et al.[11], using bovine serum albumin fraction 5 as standard.

All solutions were made up in glass-distilled, deionized water. All inorganic salts were AnalaR grade or the best commercially available; histidine, ATP, bovine serum albumin fraction 5, and ouabain were obtained from Sigma Chemical Co.; ATP (Tris salt) was made from the sodium salt by ion exchange. Cirrasol ALN-WF was a gift from ICI Dyestuffs Division.

Results. The effect of temperature on the ouabain-sensitivity of fluid secretion by the Malpighian tubules was such that at temperatures below 30 °C ouabain-inhibition decreased. Thus at 30 °C, $56 \pm 6.8\%$ ($n = 28$) inhibition was observed whereas at 20 °C the level of inhibition was only $28 \pm 5.5\%$ ($n = 30$) and at 15 °C no significant reduction in the rate of secretion was observed ($4.1 \pm 5.7\%$ inhibition; $n = 27$). The 100% rates of secretion were 4.2 ± 0.5 nl/min at 30 °C, 2.2 ± 0.2 nl/min at 20 °C and 2.2 ± 0.3 nl/min at

The effect of temperature on the inhibition of Na^+-K^+-activated ATPase activity by 1 µM ouabain

Temperature (°C)	Experiment No.	Activity nmoles Pi liberated/mg protein/min		% Inhibition
		+ Ouabain	− Ouabain	
30	1)	130.2	285.7	54.5
	2)	159.1	292.1	45.6
20	1)	86.5	138.6	37.6
	2)	112.3	155.0	27.6
10	1)	43.3	51.7	16.3
	2)	57.9	67.4	14.1

15 °C. In contrast to the effect of temperature, changing the K^+ concentration of the bathing medium had no significant effect on the ouabain-sensitivity of fluid secretion, despite the fact that the rate of fluid secretion, itself, increased with increasing K^+ concentration. The mean rates of fluid secretion (in the absence of ouabain) were 3.4 ± 0.9 nl/min ($n=22$) in the presence of 10 mM K^+, 4.4 ± 0.7 nl/min ($n=25$) in the presence of 20 mM K^+ and 7.3 ± 1.0 nl/min ($n=21$) in the presence of 40 mM K^+. At all concentrations of K^+, the level of ouabain-inhibition of fluid secretion was about 57%.

The results presented in the table show that the inhibition of Na^+-K^+-activated ATPase activity by ouabain decreases as the temperature is lowered. At 30 °C, enzyme activity was inhibited by about 50% whereas at 20 and 15 °C the levels of inhibition were about 32% and 15% respectively.

Discussion. As mentioned briefly in the introduction, a number of researchers have failed to demonstrate an inhibitory effect of ouabain on the Malpighian tubules of *Calliphora*[12], *Rhodnius*[13], *Carausius*[14], *Glossina morsitans*[15], *Locusta*[5] and *Zonocerus*[5]. In contrast, other workers have shown that Malpighian tubule function is ouabain-sensitive in *Locusta*[1,3], *Drosophila hydei*[16] and *Glossina morsitans*[17]. In addition, Farquharson[18] has shown that fluid secretion by the Malpighian tubules of the pill millipede, *Glomeris marginata,* is sensitive to ouabain at concentrations as low as 5 µM. One of the main differences in methodology in the literature concerns the temperature at which fluid secretion by the Malpighian tubules has been studied. Rafaeli-Bernstein and Mordue[5], who report no effect of ouabain on fluid secretion in *Locusta,* carried out their experiments at 24–25 °C. Other workers[12–15] have performed their experiments at room temperature (19–22 °C). The inhibition of Na^+-K^+-activated ATPase by ouabain has been shown to be substantially affected by temperature in the present study; confirming the results of other workers[6,19]. In addition, fluid secretion by *Locusta* Malpighian tubules was far less sensitive to ouabain at 15 and 20 °C than at 30 °C (the temperature used by Anstee and Bell[1] and Anstee et al.[3]). Temperature is clearly an important factor in determining the extent to which ouabain inhibits Malpighian tubule function in *Locusta.* This may be expected if a Na^+-K^+-activated ATPase is involved in the mechanism of fluid secretion and one must conclude that failure to demonstrate inhibition of fluid secretion at room temperature, by ouabain, should not be taken as evidence against the involvement of this enzyme in the secretory mechanism.

In the present study, varying the K^+ concentration from 10 to 40 mM failed to affect the ouabain-sensitivity of the fluid secretory process. It would seem, therefore, that the K^+ concentration of the bathing medium may vary quite substantially without effecting a significant reduction in ouabain-inhibition.

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Physical exercise stimulates marked concomitant release of β-endorphin and adrenocorticotropic hormone (ACTH) in peripheral blood in man

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Summary. ACTH and β-endorphin have been evaluated by means of a specific and sensitive radioimmunoassay in athletes reaching a status of physical stress. A concomitant marked increase of these 2 peptides has been recorded. The implications of this finding lead to the conclusion that stress stimulates the synthesis of the common precursor (31 K) in the pituitary.

Endorphins possess an analgesic action and probably produce other opiate-like effects as well, but the real physiological significance of these substances has not yet been elucidated. The fact that 2 different compartmentalized endorphin pools exist, one in peripheral blood coming from the pituitary[1,2], the other directly synthesized by the peptidergic neurons in the brain[3,4], seems to indicate different biological actions of these substances, one with a direct effect on the neurons in the CNS, another with a hormonal effect at peripheral level. So far only an analgesic effect, and no other physiological significance, has been demonstrated. Whether or not the peripheral effects, subsequent to pituitary release, are mediated by a target organ remains to be elucidated. Furthermore, it is not yet known whether a specific release mechanism exists in the brain or pituitary.

Stress may play a role in releasing these peptides, thus indicating a common biosynthetic pathway[5], at least in the pituitary, with that of ACTH.

Rossier et al.[6] have shown that foot-shock stress in rats leads to an increase in opioids at the peripheral level, but not in the brain, indicating a specific release mechanism for