SHORT COMMUNICATION

ANGIOTENSIN II CAN INITIATE CONTACT-REHYDRATION IN TERRESTRIAL SLUGS

BY MARTHA E. MAKRA AND DAVID J. PRIOR

Physiology Group, School of Biological Sciences, University of Kentucky, Lexington, Ky 40506, U.S.A.

Accepted 8 May 1985

Moist-skinned terrestrial animals such as snails and slugs are highly susceptible to desiccation. It has been shown that active slugs can lose up to 35\% of their initial body weight (\%IBW) within 2–3 h (Dainton, 1954; Prior, Hume, Varga & Hess, 1983). They can, however, rapidly recover from dehydration by integumental absorption of water while in contact with a moist substrate (Burton, 1966; Prior, 1982, 1984). This process of contact-rehydration involves an increase in the epithelial paracellular permeability of the foot and is mediated by a well regulated behavioural sequence (Prior, 1982, 1984; Prior & Uglem, 1984). Contact-rehydration is initiated when slugs have been dehydrated to the threshold level of 60–70\% IBW. They move onto a moist surface and assume a characteristic flattened posture while water is absorbed through the foot. They remain in this posture until enough water has been absorbed to achieve their rehydration set-point (93.6 ± 12.2\% IBW for *Limax maximus*; Prior, 1984). Once rehydrated, the slugs move off the moist surface and come to rest on a dry surface, thus terminating the behaviour.

It has recently been shown that the control of both initiation and termination of contact-rehydration in slugs involves specific changes in haemolymph osmolality that accompany variations in body hydration (Prior et al. 1983; Prior, 1984). In *Limax maximus* the behaviour is initiated when the osmolality of the haemolymph is increased from the normal 140 mosmol kg\(^{-1}\) \(H_2O\) at 100\% IBW to about 200 mosmol kg\(^{-1}\) \(H_2O\) by either air-dehydration (to 70–60\% IBW) or by injection of a hyperosmotic mannitol solution. In turn, the behaviour is terminated when haemolymph osmolality is lowered, by either contact-rehydration or injection of dilute saline (Prior, 1984).

The similarities between the control of contact-rehydration in slugs and osmometric drinking in vertebrates (e.g. Andersson, 1978; Fitzsimons, 1979, 1980) led us to examine the possibility that the 'vertebrate drinking hormone', angiotensin II (AII), could initiate contact-rehydration. The present results indicate that release of an 'AII-like' peptide may be involved in the control of both initiation of the behavioural sequence and the increase in integumental water absorption that are characteristic of contact-rehydration.

Key words: Contact rehydration, slugs, angiotensin II.
Specimens of *Limax maximus* were kept in vented plastic boxes lined with moist paper towels. They were fed rat chow *ad libitum* and maintained in a growth chamber in a 14:10 h L:D cycle, at 18°C during the light period and 12°C during the dark period. Before each experiment, slugs were fasted for 5–7 days in high humidity conditions to establish a stable relationship between body weight and hydration (see Prior *et al.* 1983). This allowed the use of '% of initial body weight' as a measure of relative body hydration.

Slugs weighing 2–3 g were air dehydrated at room temperature (18–22°C) and relative humidity (20–55%) in dry mesh covered plastic containers. In each experiment the behavioural responsiveness of the slugs was tested by placing them in a dry plastic Petri dish (15 cm diameter) with a small pad of tissue paper saturated with distilled water. If a slug did not move directly onto the moist pad and assume the characteristic posture, it was removed and retested 15 and 30 min later. Slugs were weighed before dehydation (i.e. 100 % IBW), after dehydation (to 80 % IBW) and when they voluntarily moved off the wet pads. Angiotensin II (human form: Sigma) and a saralasin analogue (Sar¹Ala⁸Angiotensin II, Sigma), were injected through the posterior body wall into the haemocoel. The injections were calculated to result in final haemolymph concentrations of 5 x 10⁻⁹ mol⁻¹ All and 5 x 10⁻⁷ mol⁻¹ saralasin (see Prior, 1983). Each slug received both control isosmotic saline injections and experimental injections over a 2-week period. The rate of water absorption was calculated by the method of Prior *et al.* (1983) and statistical analyses were done with an ANOVA procedure, Duncans Multiple Range Test and Least Square Means.

It has been shown that injection of All (5 x 10⁻⁵) mol⁻¹ into fully hydrated slugs results in a low level of integumental water absorption (1·0 ± 0·4 μl cm⁻² min⁻¹), but not initiation of the contact-rehydration behaviour (Makra & Prior, 1983). However, Table 1. Effects of angiotensin II and subthreshold dehydration on contact-rehydration in *Limax maximus*

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Number of slugs that initiated the behavioural posture</th>
<th>Mean rate of water absorption (μl cm⁻² min⁻¹ ± S.E.M.)</th>
<th>Mean rehydration set-point (% IBW ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal air-dehydration to 65% IBW (Prior, 1984)</td>
<td>36/39</td>
<td>7·8 ± 0·3 (N = 119*)</td>
<td>93·6 ± 1·1 (N = 119)</td>
</tr>
<tr>
<td>All (5 x 10⁻⁹ mol⁻¹) injected into slugs at 80% IBW</td>
<td>29/29</td>
<td>7·2 ± 0·9 (N = 18†)</td>
<td>92·8 ± 1·4 (N = 29)</td>
</tr>
<tr>
<td>All (5 x 10⁻⁹ mol⁻¹) and saralasin (5 x 10⁻⁷ mol⁻¹) injected into slugs at 80% IBW</td>
<td>11/29</td>
<td>7·0 ± 1·1 (N = 7†)</td>
<td>95·2 ± 3·2 (N = 11)</td>
</tr>
<tr>
<td>1·0× saline injected into slugs at 80% IBW</td>
<td>7/29</td>
<td>2·2 ± 0·5 (N = 5†)</td>
<td>88·1 ± 2·6 (N = 7)</td>
</tr>
</tbody>
</table>

Calculated haemolymph concentrations of All and saralasin are given in parentheses and each dehydration level is ±2% IBW.

* This includes additional trials in which the complete behaviour was observed.

† Only those slugs which were completely on the moist pads were included in the calculation of the rates of water absorption.

The only rate of water absorption that was significantly different from that in the normal air-dehydration trial was that of the 1·0× saline injection trial. (P < 0·05 by least square means). None of the rehydration set-points observed in the experimental groups differed significantly (by any test) from that observed during normal dehydration induced contact-rehydration.
when slugs that had been dehydrated to the subthreshold level of 80% IBW, were injected with AII (5 × 10⁻⁹ mol l⁻¹), both the behaviour and integumental water absorption were initiated (Table 1). Within 2-min of the injection of AII, 27 of 29 slugs assumed the characteristic posture on the moist pads. The remaining two initiated the behaviour at the 15 min trial. Water was absorbed by these slugs at a rate of 7.2 ± 0.9 μl cm⁻² min⁻¹ which is comparable to the absorption rate observed during normal dehydration-induced contact rehydration (i.e. 7.8 ± 0.3 μl cm⁻² min⁻¹; Table 1). In addition, the rehydration set-point of the AII injected slugs did not differ significantly from that observed during normal contact-rehydration (Table 1). Thus, the combination of subthreshold dehydration and exogenous AII can initiate the complete contact-rehydration response. A similar ‘additivity’ of stimuli is likewise a feature of the control of drinking behaviour in various vertebrate species (e.g. Fitzsimons, 1979).

Injection of a combination of AII and saralasin (1:100 ratio; Malvin, Mouw & Vander, 1977) resulted in contact-rehydration in only 11 of the 29 slugs. Neither the mean rate of water absorption (7.0 ± 1.1 μl cm⁻² min⁻¹) nor the mean rehydration set-point (95.2 ± 3.2% IBW) of the responding slugs differed significantly from those which were observed following injection of AII alone (Table 1). Thus, although saralasin was effective in blocking the behavioural response in 62% of the slugs, those that did respond displayed a rate of water absorption and a rehydration set-point that were comparable to those resulting from AII injection.

Behavioural posturing is not usually observed in slugs dehydrated to only 80% IBW (see Prior, 1985), but in this series of experiments injection of 1·0× saline together with dehydration to 80% IBW resulted in behavioural posturing in 7 of the 29 slugs. Although posturing occurred, the rate of water absorption was significantly less than that observed during normal contact-rehydration. The present results demonstrate that the combination of exogenous AII and subthreshold dehydration can initiate both the behaviour and integumental water absorption characteristic of contact-rehydration.

This research was supported by a grant from the Whitehall Foundation, and an N.I.H.-R.C.D.A. to DJP. This is contribution no. 238 from the Talahassee, Sopchoppy and Gulf Coast Marine Biological Association.

REFERENCES


