

SHORT COMMUNICATION

CHEMORECEPTION IN SEA ANEMONES:
BETAINE STIMULATES THE PRE-FEEDING RESPONSE IN
URTICINA EQUES AND *U. FELINA*

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Chemical stimulation can evoke complex behaviour patterns in sea anemones. In pre-feeding behaviour a diffusible component of food causes the oral disc to expand and the column to extend and bend from side to side. These movements may increase the chance of catching nearby food (Pollock, 1883). The response is coordinated by the ectodermal slow conduction system (SS1). In *Urticina eques*, food extracts that excite the pre-feeding response evoke a low-frequency series of SS1 pulses; and electrical stimulation of the SS1, in the absence of food, causes pre-feeding movements (McFarlane, 1970). The SS1, the SS2 (an endodermal slow system) and a through-conducting nerve net (TCNN) are the three known conducting systems that appear to coordinate sea anemone behaviour (McFarlane, 1982). Other conducting systems have been proposed (e.g. Lawn & Ross, 1982) but not confirmed.

Although the SS1 plays an important part in the coordination of many behaviour patterns, no chemical that stimulates it has been identified. We have investigated SS1 excitation during the pre-feeding response of *Urticina felina* and *U. eques* (previously known as *Tealia felina* var. *coriacea* and var. *lofotensis*, Manuel, 1981), using the technique developed by Lawn (1975). We have screened many possible activators, chosen from published analyses of known stimulatory foods, e.g. muscles from fish (Long, 1961), *Nephrops* (Robertson, 1961) and *Mytilus* (Bricteux-Grégoire, Duchâteau-Bosson, Jeuniaux & Florkin, 1964).

Anemones were bisected longitudinally, pinned cut edge down, and allowed to recover for 24 h. Test solutions were either applied in the bath to give a known final concentration or were drawn into a suction electrode (2 mm internal tip diameter) that was then attached to the column. Suction electrodes on tentacles were used to record electrical activity in all three conducting systems. As spontaneous SS1 pulses were rare, responses to the applied chemical were obvious.

The chemicals tested, at concentrations between 10^{-6} and 10^{-1} mol l⁻¹ (final bath concentration or concentration in electrode) were AMP, alanine, arginine,

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asparagine, aspartic acid, betaine, carnitine, cysteine, cystine, glutamic acid, glutamine, reduced glutathione (GSH), glycine, histidine, hypoxanthine, inosine, isoleucine, lactic acid, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, taurine, threonine, trimethylamine, trimethylamine oxide (TMO), tryptophan, tyrosine and valine. Of these, betaine (trimethyl glycine) was the most effective SS1 stimulant (Fig. 1). We conclude that betaine is the normal pre-feeding stimulant, as intact anemones, exposed to $10^{-2} \text{ mol l}^{-1}$ betaine, displayed typical pre-feeding movements. Both carnitine and TMO evoked some SS1 activity, but were always less effective than betaine. These results suggest that the receptors detect the $(\text{CH}_3)_3\text{-N}$ moiety. No other substance excited the SS1 although some

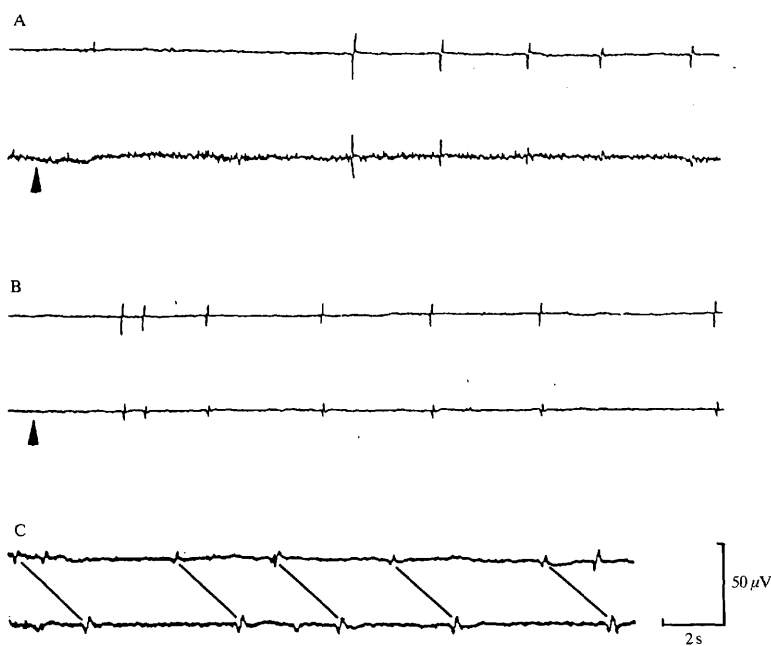


Fig. 1. SS1 pulses evoked by betaine and by food in *Urticina felina* and *U. eques*. Pulses were recorded by two suction electrodes attached to different tentacles. (A) SS1 pulses following direct application (at arrowhead) of *Mytilus* extract to the column of a half-animal preparation of *U. felina*. (B) SS1 pulses following direct application of $10^{-2} \text{ mol l}^{-1}$ betaine to the column of the other half of the same preparation. (C) SS1 pulses recorded from *U. eques* after a suction electrode containing $10^{-2} \text{ mol l}^{-1}$ betaine was attached to the column. As this was a large animal and the two recording electrodes were on widely separated tentacles it is easy to see that all evoked SS1 pulses must arise at the same point. Lines connect the same pulse seen at the two recording sites. Unmarked pulses are spontaneous SS2 pulses.

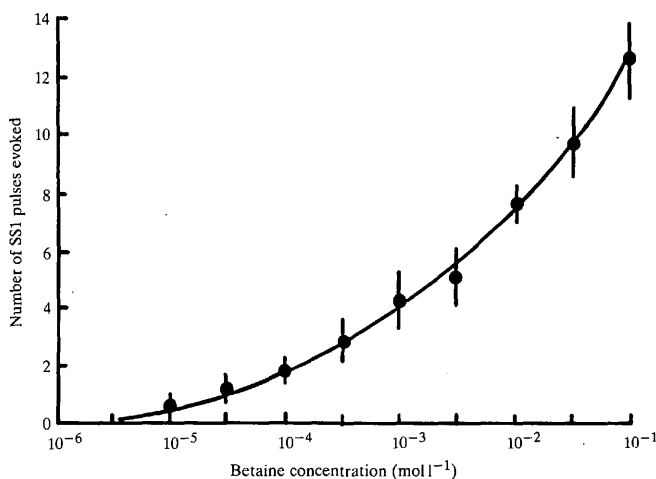


Fig. 2. Dose/response curve for the betaine response in *Urticina eques*. Ten different preparations were used and the solutions were applied in suction electrodes attached to the column. The response is recorded as the number of SS1 pulses evoked in the 2 min period after electrode placement. Each point is the mean of between 7 and 18 trials; vertical bars show standard error.

(proline in particular) stimulated the SS2 when placed in the bath, but not when applied to the column. There is some evidence that the SS2, although an endodermal conducting system, has ectodermal receptors in the tentacles and pharynx (McFarlane, 1975). GSH, in the bath, excited TCNN pulses.

Betaine was only effective when the electrode containing it was attached to the column: the other ectodermal regions tested, the tentacles and oral disc, were insensitive. The observed distribution of chemoreceptive sites agrees with the results obtained by Lawn (1975). The receptors appear to be widespread on the column but their nature is not known. Most properties of the SS1 are consistent with a neural basis for the conducting system but a complete nerve net has not been described in the column ectoderm. In *Metridium senile* scattered sense cells are, however, present in this position (Batham, Pantin & Robson, 1960) and we propose that such cells are the SS1 chemoreceptors.

A dose/response curve for the action of betaine on the SS1 (Fig. 2), based upon the activity evoked during the first 2 min of the response, shows that the SS1 chemoreceptors are not particularly sensitive. High betaine concentrations, ranging from 10^{-2} to $1.5 \times 10^{-1} \text{ mol kg}^{-1}$ tissue water, are, however, found in most marine animals (Burton, 1983). The concentrations present in known pre-feeding activators are: *Mytilus* muscle, $5 \times 10^{-2} \text{ mol l}^{-1}$; *Homarus* muscle, $1.5 \times 10^{-2} \text{ mol l}^{-1}$; and fish

muscle, $10^{-2} \text{ mol l}^{-1}$ (Bricteux-Grégoire *et al.* 1964; Beers, 1967; Long, 1961). These values, taken together with the dose/response curve, suggest that food must be close to the column to excite the chemoreceptors. Unlike *Urticina eques*, *U. felina* is a littoral species and, as high concentrations of activators might occur in tide pools, close proximity of food might not always be necessary for pre-feeding behaviour to be evoked.

It is not known how many SS1 pulses are required to elicit the pre-feeding behaviour. Although the SS1 sensory response was normally complete within a few minutes, cycles of column extension and bending could persist for several hours. Possibly the SS1 causes long-term activation of phases of nerve net activity, as is known to occur in *Calliactis parasitica* (McFarlane, 1983).

This is the first time an identifiable chemical has been shown, by electrophysiological techniques, to excite the SS1. Other chemically induced, SS1-coordinated, behaviour patterns include shell-climbing behaviour in *Calliactis parasitica*, excited by mollusc shells (Ross & Sutton, 1961; McFarlane, 1976) and swimming in *Stomphia coccinea*, evoked by certain starfish and nudibranchs (Yentsch & Pierce, 1955; Robson, 1961; Lawn, 1976). In neither case, however, has the stimulant been identified. Some activators of true feeding behaviour in anemones are known (Lindstedt, 1971): tentacle contraction and mouth opening are triggered by amino acids, such as proline, glutamic acid and asparagine, or by the tripeptide reduced glutathione (GSH). Proline and GSH, applied to the mouth of *C. parasitica*, evoke SS2 pulses that lead to mouth opening and pharynx protrusion (McFarlane, 1975).

The only other identified excitatory chemical is the alarm pheromone, anthopleurine, which is released by wounded *Anthopleura elegantissima*, and causes fast withdrawal contractions of nearby conspecifics (Howe, 1976a). In *C. parasitica* it is the TCNN that normally coordinates fast and slow muscle contractions triggered by mechanical stimulation or endogenous pacemakers (McFarlane, 1982); the alarm reaction may be a rare example of chemical excitation of the TCNN. Interestingly, like betaine, anthopleurine is a quaternary ammonium compound. The anthopleurine response differs from the betaine response, however, in two main ways. First, the anthopleurine receptors are extremely sensitive: they respond to concentrations as low as $10^{-9} \text{ mol l}^{-1}$. Secondly, anthopleurine receptors are on the tentacles, not on the column; this is to be expected as in all species so far studied the TCNN is found in the tentacle ectoderm but not the column ectoderm (Batham *et al.* 1960).

Anthopleura elegantissima also shows pre-feeding behaviour and Howe (1976b) suggested, but without supporting electrophysiological evidence, that proline was the stimulant. In *Urticina eques* and *U. felina* proline excites the SS2 but not the SS1. In both these species the SS1 responds to betaine: this may reflect their close taxonomic relationship. It remains to be seen if other anemones employ other pre-feeding activators.

In some decapod crustaceans the quaternary ammonium compounds betaine and TMO have been shown to stimulate receptors upon the dactyls and effect the orientation phase of feeding behaviour (Laverack, 1963).

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