the average age of onset was 36 years (see figure) compared to an average age of onset of 50 years for other SOD-1 patients with familial ALS[6]. This indicates that damage is occurring more rapidly in these individuals and suggests both that superoxide radicals are involved in the disease mechanism and that motor neurons are vulnerable to relatively small changes in the levels of SOD activity.

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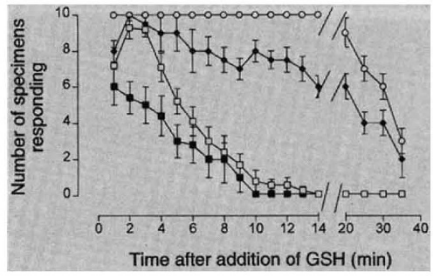
NO in hydra feeding response

SIR — In studying *Limulus polyphemus*, the horseshoe crab that has not evolved in 500 million years, Moncada and his group have observed, surprisingly, that the nitric oxide (NO) pathway may have been preserved throughout evolution[1]. Recently, NO synthase has been reported to be present in different invertebrate groups[2]. Here we have investigated whether the NO pathway is present in *Hydra* (phylum: Coelenterata), the most primitive invertebrate with a nervous system.

We have shown, using ^{3}H-citrulline generation from ^{3}H-L-arginine, that *Hydra vulgaris* express NO synthase activity. In hydra homogenates, ^{3}H-citrulline production is dependent on the presence of NADPH and is significantly inhibited by preincubation with L-N^{ω}-nitroarginine methyl ester (L-NAME; 100 μM), a specific NO synthase inhibitor. Moreover, when resuspended in EGTA–Ca^{2+}-free buffer, hydra homogenates incubated with 1 mM NADPH show a significant decrease ($P \leq 0.001$) in NO synthase activity, thus indicating that the NO synthase isoform is Ca^{2+}-dependent.

By measuring nitrite (NO_2^-), the breakdown product of NO, we find that hydra are able to release NO. Reduced glutathione (GSH; 2.5 μM), an activator of hydra feeding response (see below), enhances levels of NO_2^- (2–3-fold compared with basal levels; $P \leq 0.001$), as measured by the Griess reaction; this effect is abolished by L-NAME (100 μM).

We were astonished to find that NO is also involved in regulation of the hydra feeding response, which is the most primitive olfactory-like system present in a multicellular organism. The feeding response is a complex behavioural phenomenon consisting of tentacle writhing and mouth opening; it is elicited by the outflow of GSH from the prey itself when pierced by tentacle nematocysts[3]. The hydra feeding response arises a few seconds after GSH (2.5 μM) addition, reaches its peak within a very few minutes and gradually disappears in about 10 min, despite the presence of GSH in the bathing medium (see figure). The response is thought to terminate through the onset of an inhibitory mechanism[4].



GSH induces feeding response in *H. vulgaris*. GSH-induced response was analysed by recording the number of specimens showing the typical feeding response (tentacle writhing and mouth opening) each minute under a stereo-microscope. The response achieves its peak after a 2-min addition of GSH (2.5 μM) and disappears after about 10 min (controls) (□). A 24-h pretreatment with dbt^2-cGMP (100 μM) (■) significantly shortened the GSH-induced feeding response. In contrast, a 1-h pretreatment of hydra by injecting L-NAME (100 μM) (○) or L-NMMA (100 μM) (◆) into the gastric cavity prolongs the GSH-induced feeding response by up to 40 min. Each point represents mean ± s.e.m. of 10 experiments. Two-way ANOVA test was used for significant differences between treatments. $P \leq 0.0001$ between controls and dbt^2-cGMP or L-NAME or L-NMMA.

By using NO synthase inhibitors that reduce NO levels, we have demonstrated that NO is involved in the inhibition of the GSH-induced feeding response. In fact, when hydra were injected with L-NAME (100 μM) or L-N^G-monomethyl arginine into the gastric cavity and preincubated for 1 h, the GSH-induced feeding response was prolonged by up to 40 min (see figure); this effect was lost by co-incubation with L-arginine.

Furthermore, treatment of hydra with GSH (2.5 μM) induces an increase in cyclic GMP production, the maximum effect being observed after a 1-min treatment (from 114 to 285 fmol; 10 specimens; $P \leq 0.001$). This increase is abolished by preincubation of hydra with L-NAME ($P \leq 0.01$), indicating that the cGMP generation results from NO release. The involvement of cGMP in the inhibitory mechanism of GSH-induced feeding response is demonstrated by a 24-h pretreatment with N^2,2′-*O*-dibutyrylguanosine 3′:5′-cyclic monophosphate (dbt^2-cGMP; 100 μM), an analogue of cGMP, which can significantly shorten($P \leq 0.0001$) GSH-induced feeding re- sponse in untreated hydra as well as in L-NAME- or L-NMMA-treated specimens. On the other hand, we have shown previously that the phosphodiesterase inhibitors theophylline and isobutyl methylxanthine dramatically inhibit the hydra GSH-induced feeding response[5].

In conclusion, we confirm that the NO/cGMP pathway is preserved throughout evolution, in that it is involved in the most primitive model of an olfactory-like system present in a multicellular organism.

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MHC evolution

SIR — Peter Parham in News and Views[1] discussed results from Michael Brenner's group showing that CDlb, a molecule distantly related to MHC class I and class II molecules, binds a lipid antigen[2]. One point he made (among others) was that mice do not have a CDlb-like molecule and that this lipid-binding function evolved, therefore, after the separation of the mammalian orders. Although this late evolution appears to have occurred with many MHC class I-like molecules, particularly those encoded in the MHC, it does not appear to be the case for CD1. A molecule homologous to CDlb has been identified in rabbits[3,4] and similar molecules appear to exist in other species[5]. This indicates that CDlb-like genes evolved much earlier, before the human and rabbit lineages split, and that these genes were subsequently deleted from the mouse.

Interestingly, the murine CD1 locus on chromosome 3 (which contains two genes closely related to the human CDld gene) spans a putative chromosomal translocation site that split chromosome 1 (which contains the human CD1 locus) during the divergence of humans and mice[6]. This location is the evolutionary equivalent of a 'smoking gun', strongly supporting the