Search for the neurodepressing hormone in a stomatopod, *Squilla mantis*[1]

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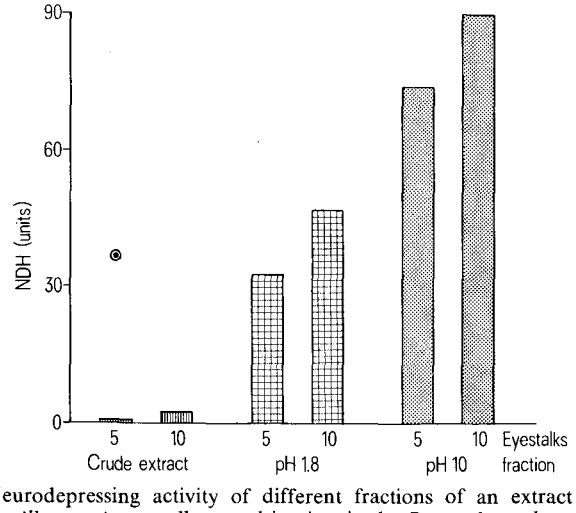
Summary. Crude extracts of *Squilla mantis* eyestalks have no neurodepressing activity in the *Procambarus bouvieri* bioassay. However, partially purified extracts show a very high level of neurodepressing hormone with the same characteristics as the decapod hormone.

It has been known since 1941[2] that eyestalk ablation in crustaceans produces an enhancement of locomotor activity and that the injection of an eyestalk extract depresses locomotor activity. These facts have led to the postulation that the release of a hormonal agent from the eyestalks results in depression of neuronal activity[3,4]. We have shown that circadian variations of rhythmic activity in the nervous system of the crab *Carcinus maenas* are modulated by a low molecular weight neuropeptide[5] which is present in the sinus gland and nervous system of the crayfish *Procambarus bouvieri*[6,7]. The isolation and purification of this neurodepressing hormone (NDH) has been accomplished in the crayfish *Procambarus bouvieri*[8] and the shrimp *Penaeus vannamei*[9]. We have recently published the results of an extensive search for neurodepressing activity in a series of marine and fresh-water decapods[10], and we have generalized that this hormone is a feature common to all decapods. In the present work we report the results of a search for the NDH in a stomatopod, *Squilla mantis*.

Materials and methods. Mature specimens of *S. mantis* were captured by one of us (A.H.) along the Pacific coast of Mexico. The eyestalks were cut immediately and kept frozen till used. 150 eyestalks were processed as described[10] for small samples and for analytical purposes. In brief, they were ground to a fine powder in a precooled mortar, using powdered solid CO_2 as abrasive; treated for 30 min with acetone at 45 °C, dried with an air current, and treated for 30 min with chloroform at 45 °C; after drying with an air current, the powdered eyestalks were extracted with 50 ml of bidistilled and deionized water (used throughout the process) under mechanical agitation for 30 min at 45 °C. The supernatant was separated by centrifugation at 900 × g for 30 min at 4 °C and decanted. This extraction was repeated 6 times and the collected supernatants (300 ml) were concentrated by rotary evaporation to 28 ml. This fraction was called 'total extract'. 18.7 ml (100 eyestalks) were dialyzed against 100 ml of water during 2 h (repeated 4 times). The collected diffusates (400 ml) were concentrated to 15 ml by rotary evaporation and this fraction was called 'crude extract'. An aliquot of 7.5 ml (50 eyestalks) was concentrated to 800 µl, centrifuged as above, and the supernatant submitted to paper electrophoresis on Whatman 3MM sheets (29 × 31 cm) at pH 1.8 (buffer: formic acid/acetic acid/water, 1/4/45, v/v/v) during 80 min at 450 V (14.5 V/cm). After drying the sheet horizontally in an oven at 80 °C, a strip 2 cm wide straddling the origin (1 cm to each side of it) was cut across, was reduced to small fragments and extracted 5 times with 1 ml water each, in spin thimbles, centrifuging each time as above. The extract was concentrated to 340 µl and was called 'pH 1.8 fraction'. 140 µl (20 eyestalks) of this fraction was submitted to paper electrophoresis at pH 10.0 (buffer: 0.1 M $(NH_4)_2CO_3$, adjusted to pH 10.0 with NH_3) as described above. The extract obtained from the origin was concentrated to 100 µl and was called 'pH 10.0 fraction'. The different fractions were submitted to bioassay in vitro[6]; the assay measures the modifications of the spontaneous firing in rate of motoneuron f-5 of the 3rd abdominal ganglion of *P. bouvieri*. A reference standard corresponding to a freshly prepared crude extract from 5 eyestalks of *P. bouvieri* was tested before and after each bioassay. The activity of NDH in the different fractions is expressed in arbitrary units. Each 1% reduction in firing rate is considered as 1 unit of activity and the upper limit is 90 units because there are spontaneous fluctuations corresponding to 10% of the average value of the spontaneous firing rate.

Results and discussion. We have usually been able to detect the presence of the NDH in total or in crude extracts at the 5-eyestalk level. In the case of the stomatopod *S. mantis* the activity was practically zero at this level and negligible at the 10-eyestalk level. These initial results were disturbing because *S. mantis* has a well-developed X-organ-sinus gland system and up to now we have correlated the presence of the NDH with the presence of a sinus gland from which the NDH is directly liberated to the hemolymph[6]. The reason for the failure to detect neurodepressing activity could be due to one of the following: a) the NDH is limited to decapod crustaceans, or b) the NDH could be present, but it would not cross-react with our decapod *P. bouvieri* bioassay on account of evolutionary divergence of the peptide's sequence. We then decided partially to purify a crude extract by our accelerated procedure[10]. To our surprise, after paper electrophoresis at pH 1.8 the extract had the same level of activity as our reference standard. Moreover, after a 2nd paper electrophoresis at pH 10.0, the activity was much higher than the corresponding reference standard (figure). We therefore



Neurodepressing activity of different fractions of an extract of *Squilla mantis* eyestalks tested in vitro in the *Procambarus bouvieri* bioassay. Each 1% reduction in firing rate of motoneuron f-5 from the 3rd abdominal ganglion is considered arbitrarily as 1 unit of neurodepressing hormone. The upper limit is 90 units which corresponds to a total blockage of the spontaneous firing rate (after deducting 10% for spontaneous fluctuations). The dot represents the activity of a reference standard consistent in a freshly prepared crude extract of 5 eyestalks of *Procambarus bouvierti*.

conclude that the eyestalk of the stomatopod *Squilla mantis* contains the neurodepressing hormone, that its NDH has the same general characteristics as the hormone obtained from decapods (a small, hydrophilic and neutral peptide), and that it cross-reacts with the *Procambarus bouvieri* bioassay. The negative results obtained with the total and crude extracts could be due to the presence of interfering substances present in the eyestalk and which are efficiently eliminated by paper electrophoresis at pH 1.8 and pH 10.0. It can be stated now that the NDH is present not only in decapods but also in the more primitive stomatopods. This speaks in favour of an evolutionary stability with general conservation of its structure, commensurate with its physiological importance as a modulator of crustacean nervous activity. In view of the above, we suggest that for analytical purposes our accelerated procedure should be used directly to detect the presence of the neurodepressing hormone in hitherto untested species.

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Reserpine prevents goldthioglucose hypothalamic lesions in mice

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Summary. In reserpinized mice the occurrence of goldthioglucose hypothalamic lesions was significantly lower than in control mice. Some protection was also conferred by serotonin-receptor blockers and by treatment with nialamide + DL-α-methyldopa, but the protective effect of reserpine was not reversed by serotonergic and dopaminergic agonists, alone or in combination, nor by insulin.

When injected into mice, goldthioglucose (GTG) accumulates in the ventromedial hypothalamus (VMH) and destroys it[1]. This makes the mice eat more, and they become obese[2]. Diabetic mice are protected from GTG lesions[3], but insulin restores their vulnerability[4]. This is one of the main arguments in favour of the glucostatic theory of feeding which holds that feeding is inhibited by the VMH when enough glucose enters it[5-7]. It has been shown, however, that treatments other than with insulin, including certain mildly stressful procedures[8], may restore vulnerability to GTG in diabetic mice[9,10]. Further investigations in this laboratory have suggested a possible role for monoamine release in this phenomenon since the immunity of the diabetic mouse appeared to be enhanced by pretreatment with reserpine, a drug known to deplete brain monoamines[11].

In the present study we have further investigated the interaction of GTG and reserpine by examining their effects in normal, non-diabetic mice. In addition we examined the effect of other agents, affecting monoamine function in a more specific manner than reserpine, on GTG lesions.

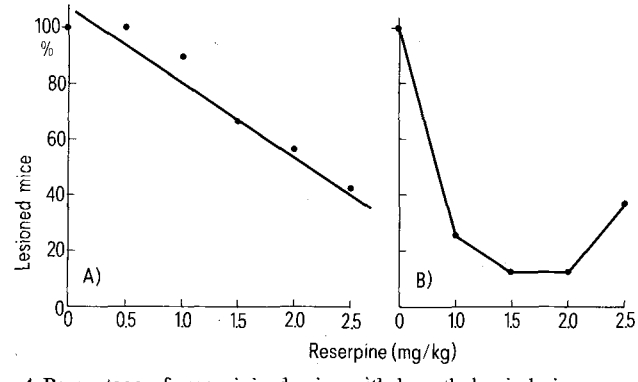
One of 2 doses (250 or 450 mg/kg, i.p.) of GTG was administrated to each mouse after treatment with reserpine or after administration of a specific synthesis - or receptor-blocker of monoamine transmitter. Hypothalamic lesions were assessed histologically in the brains of mice that had survived 3 days, as described elsewhere[10].

The protective effect of reserpine from GTG lesions can be seen in the figure A, which shows a linear dose-effect relationship. The protection was effective with 450 mg/kg of GTG, a much higher dose than ED_{100} which, in this batch of GTG and strain of mice, was of 250 mg/kg (see

Protective effect of $SQ_{10,631}$ against lesions produced by 250 mg/kg of GTG in the VMH of mice. $SQ_{10,631}$ was injected 2 h before GTG. Values are the number of mice

$SQ_{10,631}$ (mg/kg)	Total surviving mice	Lesions Yes	No
0	22	22	0
100	10	6	4
130	13	10	3
160*	5	3	2

$X^2 = 10.0$ ($p < 0.05$); $r = 0.46$ ($p < 0.01$). * LD_{50} (E.R. Squibb & Sons, Inc.; personal communication). $SQ_{10,631}$ was a gift from S.J. Lucania, The Squibb Institute for Medical Research, Princeton, N.J.



A Percentage of reserpinized mice with hypothalamic lesions produced by 450 mg/kg of GTG plotted against the dose of reserpine given 24 h prior to GTG. Regression line is $Y = -25X + 107$; $r = -.97$; $p < .001$. *B* Same results after only 250 mg/kg (ED_{100}) of GTG. Hypothalamic lesions were determined as reported in detail elsewhere[10] by photography of histological sections of the brains of the mice surviving 3 days.