ACX. Also, it seems that glucagon may play a role in controlling the activity of the protein inhibitor at the in vivo level; however, isolated liver cell studies indicated that neither protein inhibitor nor ACX are under the influence of glucagon. The decrease in ACX activity and the concomitant increase in the inhibitor activity after glucagon injection in rats may be explained by the presence of interfering factor(s).

This study as well as our earlier studies[16,19,20], suggest that ACX is not regulated via covalent phosphorylation or allosteric modulation, but more probably by the presence of other factors such as regulatory protein(s). We therefore believe that more attention should be directed to the investigation of other enzymes, particularly those which are believed to be regulated via phosphorylation where this has been demonstrated in vitro but it is not proven that regulation functions by this mechanism in vivo.

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Cardioactive peptides of the CNS of the pulmonate snail *Lymnaea stagnalis*

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Summary. In the pulmonate snail *Lymnaea stagnalis* the cardioactive effects (tested on isolated auricles) of acetylcholine (ACh), 5-hydroxytryptamine (5-HT), the bivalve tetrapeptide FMRFamide, and of chromatographically separated snail brain substances have been established. Besides ACh and 5-HT, in brain extracts, small FMRFamide-like and large cardioexcitatory peptides were found.

In molluscs the heart rate is accurately controlled[1-4]. In addition to neurotransmitters, e.g. acetylcholine (ACh) and 5-hydroxytryptamine (5-HT), cardioregulatory neuropeptides have been implicated in this control[4]. One of these, the tetrapeptide amide Phe-Met-Arg-Phe-NH_2 of the bivalve *Macrocallista nimbosa*, has recently been purified, identified and synthesized[5]. Cardioactive FMRFamide-like neuropeptides also occur in various gastropods[4,6,8]. Furthermore, from the CNS of *Helix* one or more large cardioactive peptides (LCP; 6000-8000 daltons) have been isolated[6,8]. Immunocytochemical[7] and functional[4,6,8] studies suggest that FMRFamide and related peptides function as neurotransmitters/neuromodulators, whereas LCP has a putative neurohormonal function[6,8]. In the basommatophoran *Lymnaea stagnalis* starvation causes considerable changes in kidney function, water- and ion metabolism, and acid-base balance. Very probably the decrease in heart rate plays a crucial role in these changes[3]. As part of a study of the underlying control mechanisms we plan to investigate the role of cardioactive neuropeptides. In this report, circumstantial biochemical and pharmacological evidence for the existence of FMRFamide-like peptides and LCP in *Lymnaea* CNS will be presented. Further details on the purification and characterization of these peptides will be presented elsewhere. For the in vitro bioassay we used the auricle of the heart of *Lymnaea*, as it is spontaneously active, whereas the ventricle is quiescent.

Materials and methods. The auricle of an adult, laboratory raised[9] *Lymnaea stagnalis* was dissected out, attached to a displacement transducer (designed in the institute), suspended in a 750-µl organ bath, and superfused with aerated (1.72% CO_2 in O_2) snail saline[10] (pH ~ 7.8) maintained at 20 °C, at a rate of 650 µl per min. Test substances were introduced in either of the following ways. 1. As a pulse in the superfusion fluid just before it entered the bath (dose-response curves and fractions of columns). 2. Directly into the bath; in this case superfusion was temporarily arrested (threshold determinations and pretreatments with α-bungarotoxin or methysergide). Contractions were displayed on a recorder. Test drugs were dissolved in distilled water and added to the bath in 5-10-µl doses. All doses were expressed as molar concentrations in the bath. ACh chloride (Sigma), serotonin-creatinine sulphate (Merck), FMRFamide (Serva), α-bungarotoxin (Boehringer) and methysergide (gift from Sandoz) were used. CNS with adhering nerves were excised, collected in a glass homogenizer in solid CO_2, boiled (10 min) in 0.1 M acetic acid (w/v = 1/10) and centrifuged (Janetzki TH 12, 15 min, 12,000 rpm). The pellet was extracted 3 times more. The combined supernatants were lyophilized, redissolved in 0.1 M acetic acid and used for gel filtration on Sephadex G-15 at 4 °C[6]. Incubations with pronase (Merck; final concentration 250 U/ml) were carried out in distilled water at 23 °C for 4 h, and terminated by boiling (15 min). Control incubations were done without pronase.

Results and discussion. Figure 1 depicts a representative

pattern of elution of the cardioactivity of the CNS of *Lymnaea*. The cardioinhibitory material of peak 2 is assumed to be ACh, because 1. pure ACh inhibited the auricle, 2. it co-eluted with pure ACh, and 3. its effect was blocked by 10^{-6} M α-bungarotoxin (see fig. 2). Peak 4 material is presumably 5-HT, because 1. like pure 5-HT it increased the amplitude and rate of beating (fig. 2), and 2. it co-eluted with pure 5-HT. The occurrence of ACh and 5-HT in the CNS of *Lymnaea* is in accordance with previous reports on molluscs[11-14]. In addition, there is evidence that in the CNS of *Lymnaea* cardioactive peptides occur. This is based on the observation that the cardioexcitatory materials of peaks 1 and 3 were inactivated by pronase. The (presumed) peptides of peak 3 are probably FMRFamide or a related peptide or peptides, since 1. it co-eluted with pure FMRFamide (mol.wt about 600 daltons), and 2. it has similar cardioactive effects to those of pure FMRFamide (increase of heart rate and of amplitude; fig. 2). This conclusion is supported by the demonstration of FMRFamide-like immunoreactivity in CNS and various other tissues of *Lymnaea*[7].

In *Lymnaea*, as in the bivalve *Mercenaria mercenaria*[4,5], the FMRFamide cardioexcitatory actions are similar to those of 5-HT (fig. 2). However, in contrast to the findings in other molluscs[4], in *Lymnaea* the 5-HT effects could not be blocked by methysergide. Therefore, it is as yet not clear whether 5 -HT and FMRFamide effects on the *Lymnaea* heart involve separate receptor sites. FMRFamide or FMRFamide-like peptides have been demonstrated in the CNS of various molluscs[4,6] and FMRFamide immunoreactivity has also been observed in the CNS of insects, a fish and a mammal[7]. This indicates that these peptides are phylogenetically conservative. Molluscan cardiac activity is, in part, directly regulated by the cardiac nerves. The cardioactive neurotransmitters[11-14] and probably also FMRFamide and related peptides with a neurotransmitter/neuromodulator function, are associated with this type of

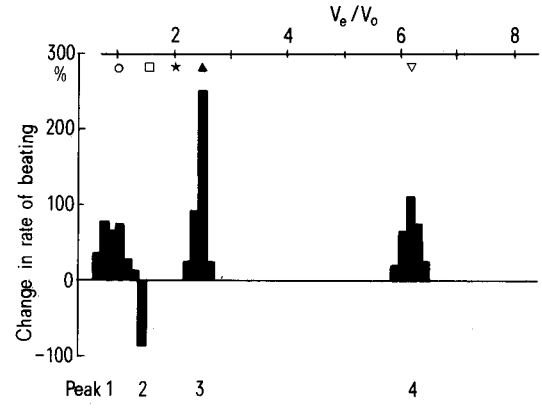

Figure 1. Cardioactivity profiles of CNS of *Lymnaea*. An extract of 450 CNS in 4 ml 0.1 M acetic acid was separated on a 90 × 1.5 cm Sephadex G-15 column at 4 °C. The eluant was 0.1 M acetic acid. The flow rate was 6.3 ml/h. Fractions (4 ml) were collected, lyophilized and taken up in 100 µl distilled water before assay. Ratios of elution volume (V_e) to exclusion volume (V_0) are indicated at the top. Markers, not run concurrently, are: ○, bovine serum albumin (mol.wt 68,000), □, ACh (mol.wt 146), *, KCl (mol.wt 75), ▲, FMRFamide (mol.wt 600), ▽, 5-HT (mol.wt 176). FMRFamide and 5-HT are retarded on Sephadex G-15 columns[6].

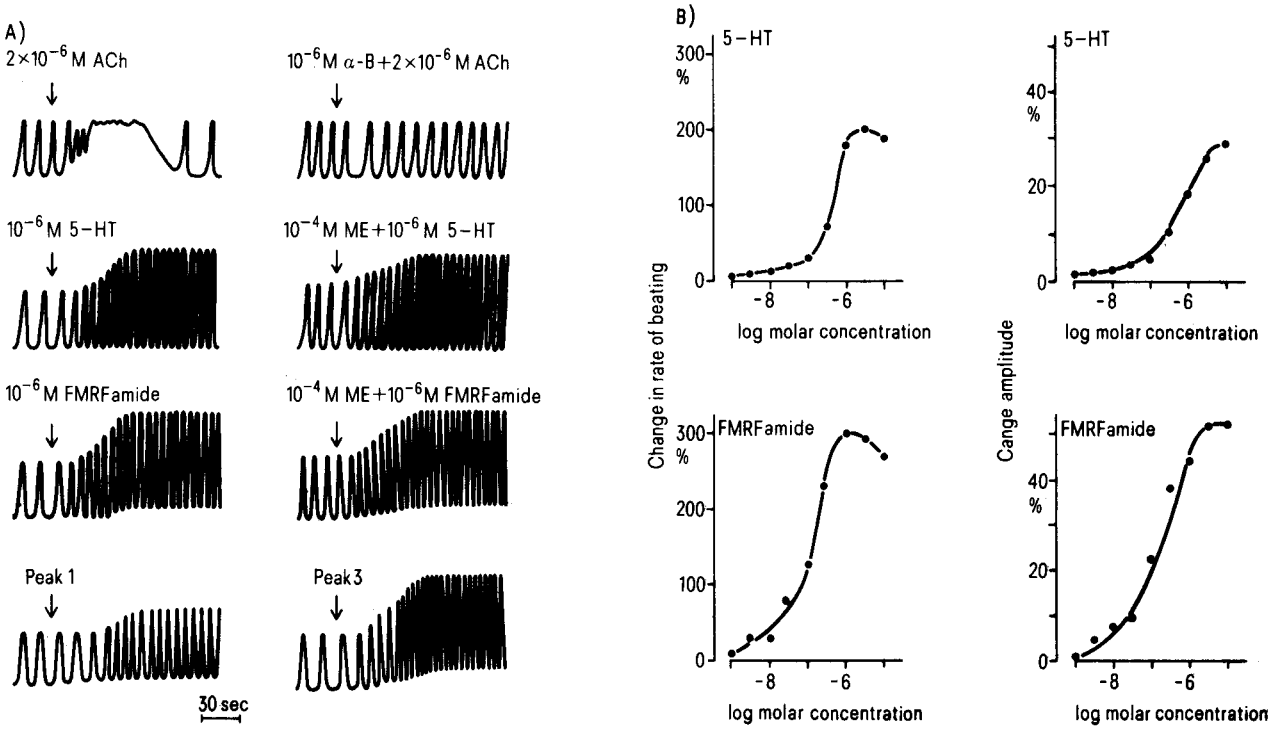

Figure 2. *A* Effects of various substances on in vitro activity of a *Lymnaea* auricle preparation. Peaks 1 and 3 represent cardioexcitation of (presumed) peptide material from fractions obtained after gel filtration of brain extracts (see fig. 1). In some cases auricles were pretreated for 20–30 min with the antiacetylcholinergic agent α-bungarotoxin (α-B) or the antiserotonergic agent methysergide (ME). Substances were added at the arrows.

B Log dose-response curves for 5-HT and FMRFamide. The responses are from one auricle and were quantified as follows. The frequency (contractions per min) and amplitude of beating were measured 2 min after addition of the test drug. Then the percentage increase-decrease over the control frequency and amplitude, measured immediately before treatment, were computed. See 'materials and methods' section for further details.

control. In many cases, however, the changes are mediated by blood constituents, including hormones, like the LCP of *Helix*. We have found indications that in the CNS of *Lymnaea* LCP (mol.wt $\geqslant$ 1500 daltons) is also present (peak 1, see figs 1 and 2A). Further studies are needed to elucidate its function.

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Is the vasospasm following subarachnoidal hemorrhage an immunoreactive disease?

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Summary. Subarachnoidal hemorrhage (SAH) from an arterial aneurysm is often followed by vasospasm which may lead to severe or even fatal ischemic brain lesions. The cause of the vasospasm is still unknown. In the present study it is shown that patients with SAH and roentgenological and/or clinical vasospasm have a significantly higher frequency (52%) of circulating immune complexes in the blood than patients with SAH without spasm (9%). This finding indicates that the vasospasm following SAH may be elicited via an immunoreaction.

The vasospasm following a subarachnoidal hemorrhage (SAH) from an arterial aneurysm often has a biphasic course: a 1st stage with vasospasm of short duration, in connection with the bleeding, and a 2nd stage with long-lasting vasospasm which appears about 1 week after the bleeding. The first vasospasm is possibly mechanically elicited while the second vasospasm is thought to have a chemical genesis. This latter long-lasting vasospasm can cause severe to fatal ischemic brain injuries. The cause of the vasospasm is still unknown. There are several studies which indicate that released blood products with vasoactive effect might cause the vasospasm. A number of such substances have been suggested, such as serotonin, histamin, plasmaproteolytic enzyme, acetylcholine, heparin and others.

The present study is based on the following theory. Antigenic substances are liberated from the site of the bleeding. These antigens are absorbed into the blood stream and will initiate an immune response with formation of antigen-antibody complexes. These immune complexes will in turn cause an inflammatory reaction at certain sites of the arterial tree with release of vasoactive substances leading to spasm. To test this thory the presence of circulating immune complexes in blood was studied in patients with SAH from a ruptured aneurysm.

Patients and methods. The study included 43 patients with SAH from a ruptured aneurysm. Blood samples were taken at varying intervals after the bleeding. Thus samples were obtained before and after surgical intervention with ligature of the aneurysm. Vasospasm was considered to be present when the patient showed angiographic signs of spasm and/or pronounced clinical symptoms indicating an ischemic lesion. There were 2 control groups. One group consisted of 20 patients with other neurosurgical diseases, operated, as well as nonoperated. The other control group consisted of 20 blood donors.

Demonstration of circulating immune complexes was performed by incubating 2×10^6 normal granulocytes prepared as described by The et al.[1] with 100 µl of the patient's serum and 50 µl of fresh normal human serum as a complement source for 90 min. Tests were considered positive only when more than 10% of the granulocytes showed immune complexes.

Results. The percentages of patients showing presence of circulating immune complexes are given in the table. Patients with spasm showed a significantly higher frequency of circulating immune complexes than patients in the other groups.

Discussion. If one accepts that the method used demonstrates the presence of immune complexes, then the frequency of circulating immune complexes is significantly higher in patients with SAH with vasospasm than in patients with SAH without vasospasm. In the latter group the frequency is as low as in the control group consisting of

The percentages of patients showing the presence of circulating immune complexes

	Number of patients	Circulating immune complexes (%)
SAH with spasm	21	52
SAH without spasm	22	9
Other neurosurgical diseases	20	10
Blood donors	20	0