Physiology

Cardiac peptides and the control of sodium excretion

from G. A. Sagnella and G. A. MacGregor

PHYSIOLOGICAL experiments have repeatedly suggested the existence of natriuretic substances that increase the excretion of sodium from the kidney, probably by inhibiting the reabsorption of sodium along the renal tubule¹. These factors are of particular interest partly because the amount of sodium in the body's extracellular fluid regulates its volume, and partly because of suggestive evidence of a link between the kidney's ability to excrete sodium and essential hypertension¹, a major risk factor for cardiovascular disease².

It has recently become clear that there are at least two distinct types of natriuretic substances. One, probably produced by the hypothalamic region of the brain, is an inhibitor of ($\text{Na}^+ + \text{K}^+$) ATPase, the enzyme which transports sodium across membranes, and might be a contributory factor to a rise in blood pressure¹. The other, comprising peptides that have been isolated from the atria of the heart, has recently become the focus of attention for numerous laboratories throughout the world, although its physiological significance remains tantalizingly uncertain. The occasion for the sudden attention to the atrial peptides is their recent characterization and synthesis; moreover, as described on pages 717 to 726 of this issue of *Nature*, their precursor has now

been identified and its sequence deduced.

Both neuronal and humoral mechanisms have been suggested³ for the increase in urinary outflow that follows stimulation of atrial 'stretch' receptors⁴. Speculation that a novel endocrine system might be involved was stimulated by the observation that intravenous injection of rats with atrial, but not ventricular, extracts led to a striking natriuretic and diuretic response⁵. Moreover, the natriuretic activity is associated with endocrine-like secretory granules of the atrial cells^{6,7}, and the number of granules changes during water deprivation⁸.

Early work suggested that the active material was a peptide⁹⁻¹² and, more recently, it was shown to be a mixture of peptides of different molecular sizes which are not only natriuretic but can also relax the smooth muscle of vascular tissue¹³, causing vasodilation. A number of peptides — variously named atriopeptins, auriculins, cardionatrin and atrial natriuretic factors — have recently been purified and sequenced¹⁴⁻²⁰; their structures are summarized in Fig.1. There are two striking conclusions. First, all the rat peptides seem to be derived from a common precursor, as suggested by earlier indirect evidence⁹⁻¹² and consistent with the isolation of a 73 amino acid peptide containing the atrial natriuretic sequence in its

carboxy-terminal region²¹. Second, the one human peptide so far sequenced differs from the rat peptide by only one amino acid. All the peptides require an intact disulphide bridge for biological activity.

The suggestion of a common precursor for the peptides is now borne out by the sequences of precursor cDNAs of both rat and human atrial peptides reported by three groups in this issue of *Nature*. Both Yamanaka *et al.*²² and Maki *et al.*²³ have cloned cDNA of the atrial natriuretic factor derived from rat atrial mRNA, and have determined its nucleotide sequence and deduced an amino acid sequence. The precursor is 152 amino acids long and contains in its carboxy-terminal region the amino acid sequences of all the atrial peptides reported to date (Fig.1). It also contains a likely signal sequence with a high content of hydrophobic amino acids in the N-terminal region, which is a characteristic feature of precursors of secretory proteins²⁴. A remarkably similar sequence is reported by Oikawa *et al.*²⁵ for the cDNA encoding a precursor of the human atrial natriuretic peptide. (The deduced sequence contains not only the human atrial natriuretic peptide α -hANP, but also γ -hANP, a 126 amino acid peptide with the α -hANP sequence at its carboxy-terminus and which Oikawa *et al.* have identified in human atrial tissue — see Fig.1). Like the rat precursor, the human precursor contains a putative signal peptide sequence at its amino-terminus. There are some differences however — in particular, the Arg-Arg dipeptide following the atrial peptide sequence in the rat precursor is lacking in the human molecule. This may reflect differences in post-translational activation mechanisms.

The physiological function and importance of the atrial peptides have yet to be defined. Clearly, the fact that they have potent natriuretic and diuretic actions on the kidney as well as a vasodilatory effect raises the possibility of a role in the regulation of body fluids and blood pressure. Moreover, the peptides antagonize the vasopressor actions of the hormones noradrenaline¹⁷ and angiotensin II¹⁶. Although limited, the available evidence is compatible with a hypothetical scheme in which an increase in extracellular fluid volume may serve as a trigger for release of atrial peptides into the general circulation (Fig.2); obviously, a number of questions remain to be answered.

How these peptides relax vascular smooth muscle and increase sodium excretion is not yet clear. They do not seem to act by inhibiting the ($\text{Na}^+ + \text{K}^+$) ATPase¹², and there is conflicting evidence as to their effect on the glomerular filtration rate — atrial extracts have no marked effect on it²⁶, whereas synthetic atrial natriuretic factor increases it slightly¹⁶. Atlas *et al.* suggest that the natriuretic effect results from the vasodilatory action of the peptide and a shift of blood flow to the medullary region of the kidney¹⁶.

The characterization of the peptides at

Fig.1 Amino acid sequences and proposed names of rat and human atrial natriuretic peptides (above) and organization of their precursors (below).

the molecular level and their subsequent synthesis provides a basis to investigate their mechanisms of action at the physiological and biochemical levels. There will undoubtedly be rapid progress in establishing radioimmunoassays to detect the presence of the peptides in the circulation and, if they are present, to test whether the circulating levels change with alteration of extracellular volume.

Clearly the potential roles of the peptides in disorders of electrolyte balance and in blood-pressure regulation remain to be clarified. For example, might Bartter's syndrome reflect excess atrial natriuretic factor and might hyporeninaemic-hypoaldosteronism be associated with a lack of it?

Finally, the relation of the atrial natriuretic peptides to other postulated, but as yet uncharacterized, natriuretic agents remains to be clarified. Teleologically, the existence of two natriuretic substances, one with vasopressor and the other with vasorelaxant properties, could allow the integrated control of both electrolyte and blood pressure homeostasis. □

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G.A. Sagnella and G.A. MacGregor are in the Blood Pressure Unit, Charing Cross Hospital Medical School, London W6 8RF.

Optical physics

Generation of the shortest ever optical pulses

from Peter Knight

How many optical cycles do you need in a pulse for it still to be called a light wave? Ippen, Fujimoto and Weiner at MIT have just reported the generation and measurement of 16 femtosecond (1.6×10^{-14} s) optical pulses of centre wavelength 620 nm which contain just eight optical cycles (*Appl. Phys. Lett.* **44**, 832; 1984). They also report that they can see the colour of the light change as their high-repetition-rate laser apparatus compresses the optical pulses. These are the shortest light pulses ever generated and, since a molecule takes about 10 fs to vibrate, they open a new time-window in the study of ultrafast processes.

The generation of picosecond optical pulses has become fairly straightforward with the use of laser mode-locking. Because laser action is encouraged over as wide a frequency band as possible, many laser modes will oscillate at once. Mode-locking gives each mode a common phase so that constructive and destructive interference among the different waves leads to a regular train of pulses. Each of these pulses has a temporal width inversely proportional to the number of modes participating and thus to the laser gain bandwidth. For this reason a dye laser which can be excited over a wide frequency range can generate extremely short pulses. For example, a colliding-pulse ring geometry, developed at Bell Labs and now used by Ippen *et al.*, can generate reliable pulses of less than 100 fs duration.

The study of ultrafast phenomena requires even these short pulses to be further compressed in time. To achieve that degree of compression, Ippen *et al.* at MIT (and other groups in Bell Labs, IBM and Rochester) have stretched out the pulse frequency spectrum even more by 'chirping' the light so that the carrier frequency is swept. When this is followed by a dispersive element, the blue-shifted training edge catches up with the red-shifted leading edge and compresses the pulse.

Chirping can be produced by the nonlinear propagation of intense laser light in a liquid such as carbon disulphide. The intensity-dependent alterations in the refractive index of the liquid cause self-phase-modulation of the light. This method, proposed by Giordmaine at Bell Labs, has proved troublesome. The optical pulses can be chirped, but not in a reproducible or stable manner.

Nonlinear optics have turned to single-mode glass fibres to produce stable uniform chirping. Light from a mode-locked laser can be focused into the small-diameter (about 10^{-3} cm) core of the fibre

to give internal intensities large enough for nonlinear effects to be important. The nonlinear refractive index produces a phase-shift which depends on the instantaneous intensity. The variable phase-shift increases until the peak of the pulse arrives and then it diminishes. The instantaneous frequency shift is given by the time-derivative of the phase shift, so that frequencies in the leading part of the pulse are lowered while those in the trailing edge are raised. The size of this frequency chirp increases directly with the length of the fibre. This chirping, combined with dispersion in the fibre, is the basis of the 'soliton laser' developed by Mollenauer and Stolen at Bell Labs (*Opt. Lett.* **9**, 13; 1984). The spectrally stretched pulse, on emerging from the glass fibre, can be compressed in time using a dispersive grating pair technique which allows the blue-shifted trailing edge of the optical pulse to catch up with the red-shifted leading edge.

Ippen's new experiment uses a colliding-pulse, mode-locked, dye laser to produce a train of pulses each of 65 fs duration. Single pulses are selected and amplified to initial energies of 5 μ J with a repetition rate of 10 Hz, and then spatially filtered, collimated and focused into an 8 mm long polarization-preserving fibre, which broadens the spectrum of pulse by a factor of about four by nonlinear chirping. This is followed by a grating pair to compress the pulse to its final length of eight optical cycles. The pulses are detected by a second-harmonic auto-correlator which determines temporal length by measuring the spatial extent of the pulse of light.

In a femtosecond, light travels one-third of a micrometre, so femtosecond pulses can be used to study a whole range of ultrafast processes which, until now, have been unresolvable. In condensed matter physics, such pulses can be used to study transport processes in semiconductors, non-radiative relaxation, and molecular vibration and re-orientation. In photo-biology and photochemistry, direct observations should be possible of energy dissipation, transport and dephasing. The generation of femtosecond pulses has stretched nonlinear optics to the limit. With only eight cycles left in a pulse of visible light, any improvement would seem to demand a shift to the ultraviolet. Meanwhile, the new results of Ippen *et al.* will be eagerly applied to a wide range of transient phenomena. □

Peter Knight is a Lecturer in the Blackett Laboratory, Imperial College, London SW7 2BZ.