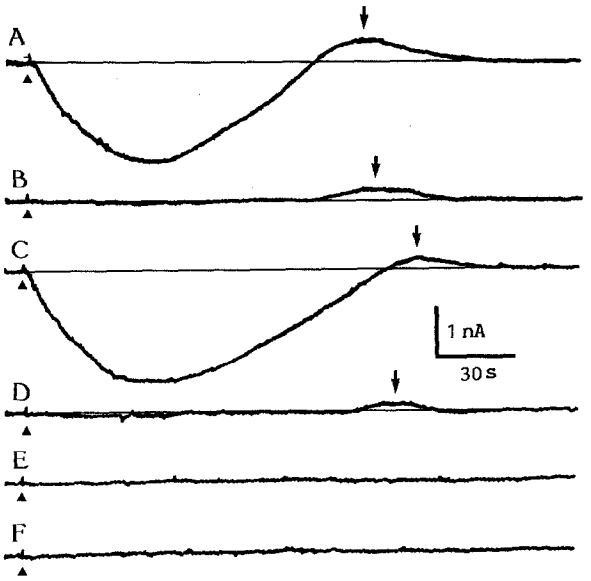

Figure 3. Inward current elicited by pressure injection of cAMP into the neurons clamped at −30 mV. *A* normal saline; *B* Na-free saline; *C* Ca-free saline; *D* Na-free, Ca-free saline; *E* 2 h after intracellular injection of PKI (1 mg/ml); *F* 1 h after extracellular application of 1.0 μM H-8. Arrow heads indicate the time of cAMP injection; arrows, outward current following inward current; and fine lines, the level of membrane current before cAMP injection into the neuron. This consecutive experiment was carried out in 10 neurons and similar results were obtained.

intracellular injection of PKC; however, Nb_{S2}-inactivated PKC, intracellularly injected in the same manner, was ineffective on the current (data not shown). This outward current is assumed to be carried by K^+ which is dependent on the released Ca^{2+} ions from the intracellular reservoir through cAMP-dependent PK, inasmuch as elevation of intracellular cAMP promotes calcium release from lysosome-like granules in *Euhadra* neurons [13] and that the current was abolished by TEA [14]. However, cAMP injection into the neuron in which PKI had been injected (fig. 3E) or into the neuron which had been treated with H-8 (fig. 3F), neither brought about any transmembrane current. These results are taken to suggest that protein phosphorylation is an indispensable step in the process leading to cAMP-mediated activation of the Na^+ current. Costa and Catterall [15] reported that the sodium channel could be phosphorylated in lyzed synaptosomes by exogenous PKC and in intact synaptosomes in the presence of 8-bromo-cAMP, which presumably acts by activating an endogenous cAMP-dependent protein kinase. Furthermore, they have observed some effects of 8-bromo cAMP-stimulated phosphorylation on the slow influx of radioactive sodium into synaptosomes. Our results are taken to conclude that protein kinase may act on the internal surface of the membrane to phosphorylate either sodium channel itself or a membrane-associated protein that enhances channel activity. Further studies will focus on elucidating the phosphoproteins involved in channel opening.

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Catch-relaxing peptide (CARP) decreases the Ca-permeability of snail neuronal membrane

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Summary. Effect of CARP was investigated on snail neurones. It was found that CARP decreased the slowly inactivating Ca- and the Ca-activated K-currents.
Key words. Catch-relaxing peptide; Ca-channel; snail neurone.

The catch-relaxing peptide (CARP) was isolated from the pedal ganglia of the mussel, *Mytilus edulis* by Muneoka and his coworkers [1, 2]. The quantitative amino acid analysis revealed the composition of the peptide which is: H-Ala-Met-Pro-Met-Leu-Arg-Leu-NH_2. The peptide relaxes catch-tension in the anterior byssus retractor muscle (ABRM) of the mussel. The ABRM is capable of eliciting a sustained contraction (catch) in response to acetylcholine or DC stimulation. The contracted muscle specifically relaxes in the presence of serotonin [3]. Twarog [4, 5] suggested that serotonin decreases intracellular free Ca-concentration and that this process is involved in the mechanisms of relaxation of the contracted ABRM. Furthermore, it was found that in addition to its catch-relaxing effect, CARP also appears to have a modulatory one on contractions in many molluscan muscles. It was speculated that the peptide plays a role in the molluscan neuromuscular system, too [2]. However, exact physiological role and mechanism of CARP action have not

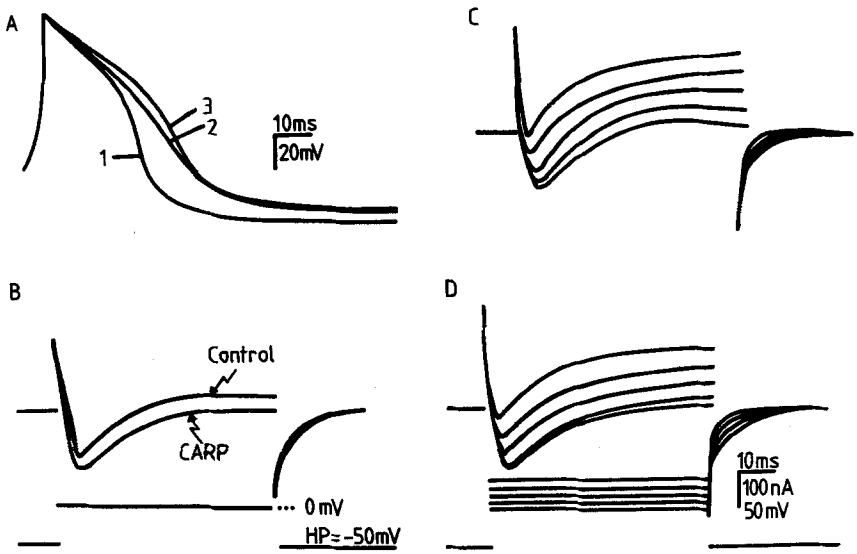

Figure 1. *A* In normal physiological saline 10 nM CARP prolonged the duration of AP (2,3) in respect to the control (1). *B* Superimposed current traces recorded at the same potentials. Examples are taken from C and D, respectively. *C,D* Total ionic currents recorded in control (C) and 10 nM CARP containing saline (D). Neurone RPa1.

yet been clarified. The aim of the experiments described in this paper was to study the effect of synthetic CARP on ionic currents of snail neurones.

Materials and methods. Electrophysiological recordings were performed at room temperature in a 0.5-ml perfusion chamber. In current clamp conditions the effects of CARP on membrane and action potentials (AP) were studied. Membrane currents were recorded using two microelectrode voltage-clamp. The 2.5 M KCl-filled microelectrodes had a tip resistance of between 1.0 and 2.5 MΩ. The cells were bathed either in physiological saline containing 80 mM NaCl, 4 mm KCl, 10 mM $CaCl_2$, 5 mM $MgCl_2$ and 5 mM Tris-HCl (pH = 7.6) or in high Ca-saline containing 50 mM $CaCl_2$, 5 mM $MgCl_2$, 20 mM TEACl and 5 mM Tris-HCl, (pH = 7.6–7.8, CaTEA saline). Experiments were carried out on identified neurones (RPa1, V4, D-cluster)[6] of *Helix pomatia* L. Neurones of D-cluster were selected because their soma membrane is almost devoid of voltage-dependent Na- and K-channels and spikes are generated mainly by Ca- and Ca-dependent K-currents[7,8]. Experiments were performed at a constant temperature (18 °C).

Results and discussion. In neurones bathed in normal physiological saline CARP was found to produce an increase of the duration of AP, and a decrease of spike after hyperpolarization (fig. 1 A). The prolongation of AP was observed both in normal physiological and CaTEA saline. The effect of CARP was reversible since after perfusing the ganglia with control saline for 5–10 min a restoration was observed. Neither the raising phase nor the amplitude of the AP changed substantially. The prolongation of AP could be a result of either an increase of the Ca-inward or a decrease of Ca-dependent K-current. In voltage-clamp conditions a decrease of outward currents was observed on the neurones bathed in normal physiological saline following the CARP administration (fig. 1 B, C, D). On the RPa1 neurone, in which the AP involves Na-, Ca-, and K-ions the inward current increased slightly, however this increase was due to the decrease of outward currents (fig. 1 B). Therefore in further experiments the effect of CARP was investigated on the Ca-inward and Ca-dependent outward currents. For this reason experiments were carried out in CaTEA saline or on the D-cluster neurones. Figure 2 shows the Ca-inward current changes occurring upon perfusion with CARP containing saline. The Ca-current amplitude decreased by about

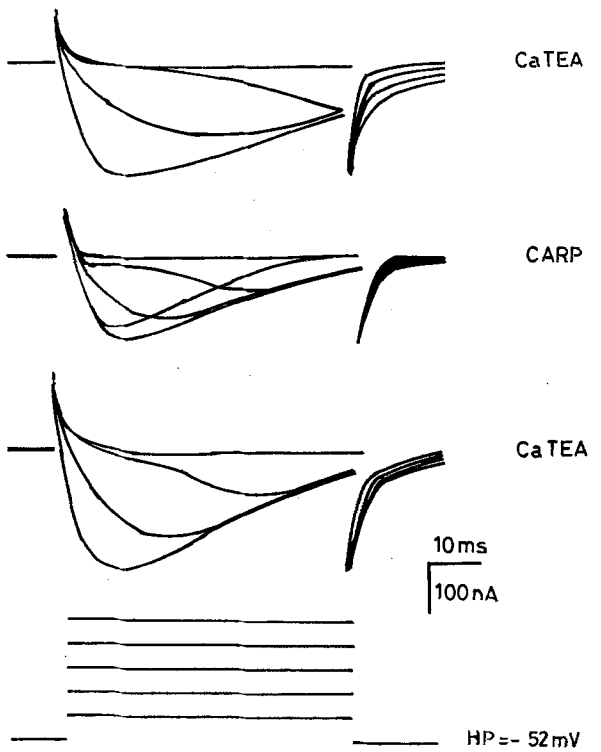

Figure 2. Family of Ca-currents in CaTEA saline (upper), in the presence of CaTEA + 10 nM CARP (middle) and after washing out with CaTEA saline (bottom) for 5 min. A substantial decrease of the slowly inactivating component can be seen comparing the current amplitudes with the control values (CaTEA) at the end of test pulses. V4 neurone.

30–40%, the time constant of inactivation changed from 53 ms to 21 ms and the tail currents amplitude decreased, too. It is suggested therefore, that CARP blocked the slowly inactivating Ca-current component (L-type Ca-channels)[9] leaving unchanged the transient Ca-current component. The outward current, which is thought to be Ca-activated potassium current was also blocked by about 30–40% (fig. 3).

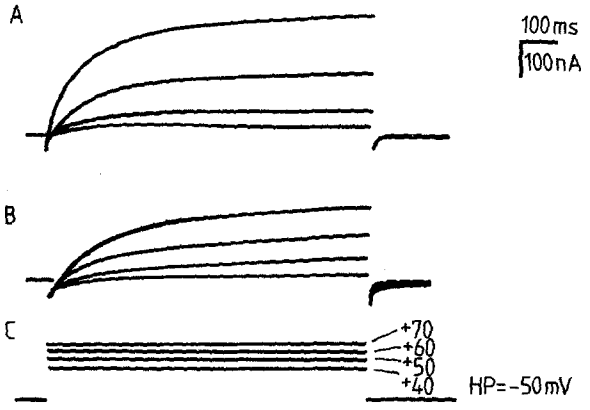

Figure 3. Comparison of Ca-dependent K-currents in control saline (A) and in the presence of 10 nM CARP (B) at different voltages. Numbers on the right to the voltage curves show values by which the neurone was depolarized from the holding potential (HP = −50 mV). D-cluster neurone.

Since both Ca-inward and Ca-activated K-currents decreased simultaneously in the presence of CARP, so that the relationship between them was linear, it is suggested that depression of K(Ca) current is merely the consequence of Ca-inward current inhibition. Recently it has became evident that neuronal membranes possess two types of voltage-sensitive calcium channels: a transient, and a slowly inactivating one. In conclusion, the data presented above suggest a specific blocking action of the CARP on slowly inactivating Ca-current component. It is proposed therefore that CARP could be a selective Ca-channel blocker.

Our experiments revealed that CARP strongly depresses the Ca-dependent K-current and the slowly inactivating Ca-inward current of snail neurones. In both cases the $(Ca)_i$ may decrease, which is consistent with the idea proposed by Twarog[4,5] for the relaxation mechanism of catch.

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Fructose-1,6-diphosphate reduces acute ECG changes due to doxorubicin in isolated rat heart

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Summary. Doxorubicin (DXR) (0.17×10^{-4} M) induces an acute cardiotoxicity in isolated rat heart; there is a progressive widening of the SαT segment, with a decrease in force derivatives and in the coronary flow. Concurrent perfusion with fructose-1,6-diphosphate (FDP) (10^{-5}–10^{-4} M) dose-dependently reduces the SαT enlargement but fails to affect the reduction in force derivatives and coronary flow. The target of cardiac protection by FDP might be the ionic mechanisms underlying the action potential configuration.
Key words. Isolated rat heart; doxorubicin; acute cardiotoxicity; fructose-1,6-diphosphate.

Doxorubicin (DXR) produces both acute and chronic cardiotoxic effects which are associated with marked ECG and hemodynamic changes in animals and in humans[1]. Various pathogenetic mechanisms have been proposed to explain the acute cardiac damage, such as free radical production[2], membrane phospholipid peroxidation[3], intracellular ATP decrease[4] and histamine release[5]. Fructose-1,6-diphosphate (FDP) is a metabolic regulator which has been successfully employed in acute myocardial ischemia[6] and in hemorrhagic shock[7], which are both characterized by a significant decrease in energy supply. Under these conditions, FDP causes a regression of electrocardiographic (ECG) ischemic changes and prevents arrhythmias in acute myocardial infarction[6,7]. In the present study, the effects of FDP on isolated rat hearts perfused with DXR are investigated. It appears from these experiments that FDP is able to reduce ECG alterations but not the contractile force changes induced by DXR.

Materials and methods. Isolated hearts. Female Sprague-Dawley rats (250–300 g) were injected with heparin 500 IU/kg i.p. and then killed by cervical dislocation. Hearts were rapidly removed and placed in a cold physiological solution; then the aorta was cannulated to allow retrograde coronary perfusion with Locke solution at 37 °C, aerated with 100% O_2, pH 7.4, in a Langendorff apparatus (perfusion pressure 60 mm Hg). Locke solution had the following composition (mM/l): NaCl 153.97, KCl 5.63, $CaCl_2$ 2.18, $NaHCO_3$ 1.78, glucose 5.09. The hearts were perfused for an initial 30-min stabilization period.

Cardiac parameters. The cardiac electric activity was recorded by a computerized on-line evaluation system to allow the measurement of various ECG parameters[8]. ECG monitoring was performed before and during perfusion with the drugs (1 h), by means of 2 atraumatic electrodes[9], one recording from the right atrium and the other from the heart apex. This system was used to measure heart rate (beats/min), the SαT segment duration (ms) and T-wave voltage (mV). The contractile function was recorded by an isometric transducer (Basile DY2) directly connected to an ADCI/T channel of a Battaglia Rangoni ESO 600 polygraph. The first derivative of the contraction or relaxation over the time ($\pm dF/dt$) was determined starting from the force signal elaborated by an AO/DP/NS operational channel (Battaglia-