pound. Azelastine has been demonstrated to show anti-PAF activities both in vitro and in vivo [15]. Moreover, it inhibits the release and synthesis of leukotrienes [14, 22]. It is likely that azelastine, due to its complex pharmacodynamic profile, is capable of inhibiting bronchial hyperreactivity in animals.

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Stimulation of sodium current by cyclic AMP is mediated through protein phosphorylation in *Euhadra* neurons

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Summary. The protein kinase inhibitors, protein kinase inhibitor isolated from rabbit muscle and isoquinolinesulfonamide, abolished the inward Na current which was elicited by cAMP.
Key words. Na current; cAMP; protein kinase; protein kinase inhibitor; protein phosphorylation; H-8; snail neuron.

There exists a surfeit of evidence on the role of cyclic AMP (cAMP) as an intracellular messenger regulating neuronal activity. As an example, recent studies have shown that intracellular cAMP injection into molluscan neurons alters membrane properties which are coupled with activation of transmembrane Na^+ inward current [1–4]. In addition, Costa et al.[5] demonstrated the selective phosphorylation of the Na^+ channel's α subunit with cAMP-dependent protein kinase (PK) and suggested that this phosphorylation may possibly be an important step in activating this channel. Recently, we demonstrated that enhancement of intracellular cAMP causes Na^+ conductance in *Euhadra* neurons[6]. Based on Greengard's theory that all effects of cAMP are mediated by protein phosphorylation [7], it is highly probable that the increment in sodium conductance may be also generated through cAMP-dependent PK which in turn, phosphorylates membrane proteins forming ionic channels in *Euhadra* neurons. In this regard, we tested the possibility that protein phosphorylation may be involved in cAMP-induced opening or activation process of Na^+ channels in snail neurons, using several pharmacological agents, the pressure injection method and the voltage clamp technique.

Materials and methods. All experiments were carried out on neurons RC-2 and RC-3 identified in the right caudal cluster of an isolated subesophageal ganglion of the snail, *Euhadra peliomphala*[6]. Procedures for dissection and the basic formula for the normal snail saline were previously described [8, 13]. The arrangements for the voltage-clamp circuitry were described elsewhere[6]. In brief, both recording and current microelectrodes, with acceptable tip resistances ranging from 1–5 MΩ, were filled with 3 M KCl. A third electrode was used for pressure injection. The membrane potential of these neurons was usually held at −50 mV with voltage step commands of 5 s duration. Clamp current was measured by a virtual ground circuit through an I–V converter. Membrane potential was kept at the holding potential (Vh) for at least 10 s before stepping to different potentials. Constant pressure (2.0 kg/cm^2) injection lasting for 1 s into the neurons was performed through an electrode with an outside tip diameter of 1–1.5 μm in which cAMP (0.1 mM), catalytic subunit of cAMP-dependent protein kinase (PKC, 1.0 mg/ml), 5,5'-dethobis (2-nitrobenzoic acid) (Nb_{S2})-inactivated PKC (1.0 mg/ml) or cAMP-dependent PK inhibitor (PKI, 1 mg/ml) was contained, as previously described [9]. Both PKC and PKI were obtained from bovine heart and from rabbit muscle, respectively, and purified in the laboratory of Dr H. Komuro (Kanagawa Dental College, Yokosuka), who also kindly provided Nb_{S2}-inactivated PKC. These were backloaded into the tip of a microelectrode. The other agent used for inhibiting cAMP-dependent PK was isoquinolinesulfonamide (H-8, Seikagaku Kogyo Co., Ltd., Tokyo) [10].

Results and discussion. PKI is a heat-stable 10-kDa protein that possesses high affinity binding to PKC and is able to block its activity [11, 12]. In the present experiment, both PKI

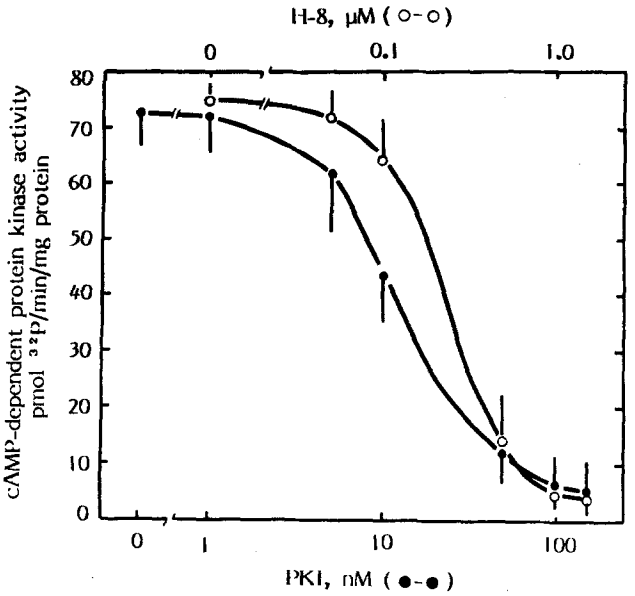

Figure 1. Inhibition of cAMP-dependent protein kinase activity by either PKI or H-8. Protein kinase activity was measured in a 12,000 × g supernatant prepared from *Euhadra* ganglion homogenates. The tissue preparation and assay conditions were as described [13]. Activity of cAMP-dependent protein kinase was measured as the difference in activity in the presence and absence of 1 μM cAMP. Basal activity measured in the absence of cAMP was 46 pmol of ^{32}P per min/mg of protein. The values on the abscissa are the final concentrations of the inhibitors in the assay. Each point is the mean ± SEM for 5–6 samples. This experiment was repeated four times with similar results.

and H-8 dose-dependently inhibited cAMP-dependent protein phosphorylation in extracts from *Euhadra* ganglia (fig. 1). Accordingly, we first tested the effect of either PKI or H-8 on the negative slope resistance (NSR) in steady state I–V curve induced by simultaneous application of 1 mM dibutyryl cAMP (db-cAMP) and 1 mM isobutylmethylxanthine (IBMX), since in *Euhadra* neurons the above agents cause the development of NSR which is dependent on $[Na^+]_o$ [6]. As seen in figure 2A, NSR was time-dependently inhibited by injecting PKI into the neuron (n = 10). In another experiment (n = 4), I–V relationships were obtained, first in normal saline after injection with PKI, then during perfusion with saline containing db-cAMP and IBMX, and finally during recovery in normal saline. PKI did not cause any shift of the resting I–V relations; however, the NSR region normally elicited by these agents was completely inhibited by PKI (data not shown). Similar experiments carried out with another inhibitor, H-8, revealed that the db-cAMP and IBMX-induced development of the NSR region was also inhibited in a dose-dependent manner by adding H-8 to bathing saline (fig. 2B). These results suggest that cAMP-dependent PK is involved in the development of the NSR region. In order to strengthen this suggestion, we also measured transmembrane current when cAMP was injected into the neurons (n = 10) held at −30 mV, since the maximum amplitude of cAMP-elicited NSR region was usually evident at this potential. These resulted in the generation of transmembrane inward current which was abolished by Na^+-free saline but not by Ca^{2+}-free saline (fig. 3A–C). A much weaker outward current following the inward current was also detected, which persisted even in Na^+-free, Ca^{2+}-free saline (fig. 3D). Similar results were obtained by

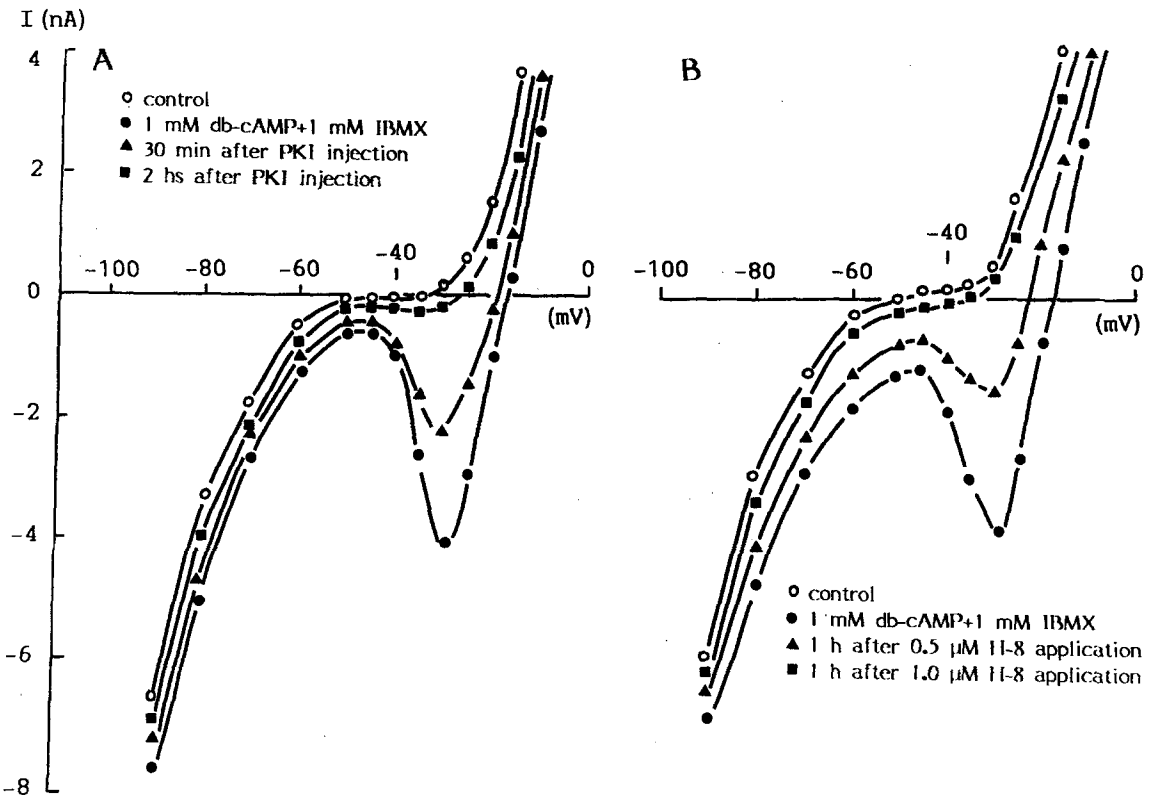

Figure 2. Effect of intracellular pressure injection of PKI (A) and extracellular application of H-8 (B) on the NSR of steady state I–V curve shifted by simultaneous application of 1 mM db-cAMP and 1 mM IBMX. Control current was measured in normal saline. Intracellular pressure injection of PKI (1 mg/ml) and extracellular application of H-8 were performed on the neuron which had exhibited the development of a NSR in the I–V curve. Vh, −50 mV. The current measurements for either intracellular injection of PKI or extracellular application of H-8 was carried out on 10 neurons, respectively.

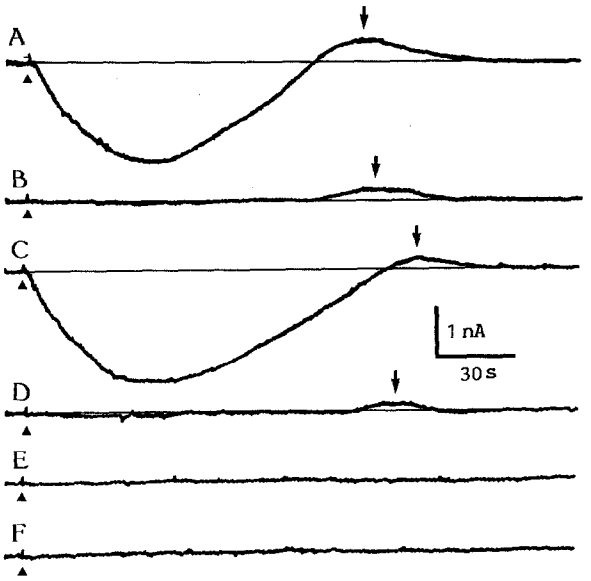

Figure 3. Inward current elicited by pressure injection of cAMP into the neurons clamped at −30 mV. *A* normal saline; *B* Na-free saline; *C* Ca-free saline; *D* Na-free, Ca-free saline; *E* 2 h after intracellular injection of PKI (1 mg/ml); *F* 1 h after extracellular application of 1.0 μM H-8. Arrow 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