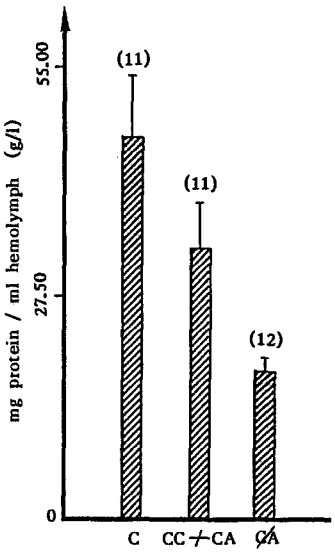

Figure 3. Hemolymph protein concentration on day-8, in day-1 allatectomized females (C/A), day-1 NCA-1 transected females (CC + CA) and control females (Control). Mean ± SEM; n, number of individual measurements.

with disconnected corpora allata show lower protein concentrations than controls but higher than allatectomized females. These results indicate that changes in concentration of JH binding sites do not reflect total protein concentration, suggesting that there is a specific control of JHBP concentration.

It is not yet clear why differences in concentration of JH-III binding sites exist, since the JHBP concentration is far in excess of the physiological JH titers[6,7]. It would also be interesting to know whether JHBP concentration is induced by changes in JH titer. Indeed, further investigations are necessary to establish the significance of JHBP fluctuation and to understand the role of JHBP in maintaining the JH titer in hemolymph.

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Calcitonin gene related peptide stimulates adenylate cyclase activity in rat striated muscle

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Summary. Rat calcitonin gene related peptide (CGRP) and salmon calcitonin (CT) stimulated adenylate cyclase activity in a dose-dependent manner in the rat diaphragm and in the kidney. The ED_{50} value of rat CGRP was lower and that of salmon CT was higher in the diaphragm than in the kidney. These results suggest that CGRP stimulates adenylate cyclase activity in the striated muscle by reacting with sites distinct from the site in the kidney.

Key words. CGRP; calcitonin; adenylate cyclase; neuromuscular junction; neuropeptide.

Amara et al.[1] showed that alternative processing of RNA transcripts from the calcitonin gene resulted in the production of distinct mRNAs encoding the hormone, calcitonin (CT), or a predicted product referred to as calcitonin gene related peptide (CGRP). Its characteristic distribution in the nervous system suggested that CGRP may be a neurotransmitter or a neuromodulator[2,3]. Recently, Takami et al.[4] have found that CGRP coexists with acetylcholine in motor neurons and nerve terminals in neuromuscular junctions of striated muscle. Furthermore, CGRP enhances muscle contraction with a concomitant increase in cyclic AMP in the tissue[5,6]. To investigate further the action of CGRP on the cyclic AMP system, we studied the effect of CGRP on adenylate cyclase activity of the striated muscle, in comparison with the effect of CT, because it has been suggested that CGRP and CT may each cross-react with the specific receptor of the other in other tissues[7].

Male Sprague-Dawley rats (Charles River Japan) weighing about 200 g were decapitated and the diaphragm and kidneys were removed. Each tissue was homogenized in 100 vols of 10 mM Tris-maleate buffer (pH 7.4) by polytron for 20 s. The homogenates were centrifuged twice at 15,000 × g for 10 min with rehomogenization of the pellet in fresh buffer. The final pellets were suspended in 99 vols of the same buffer.

Adenylate cyclase activity was measured in an incubation mix-

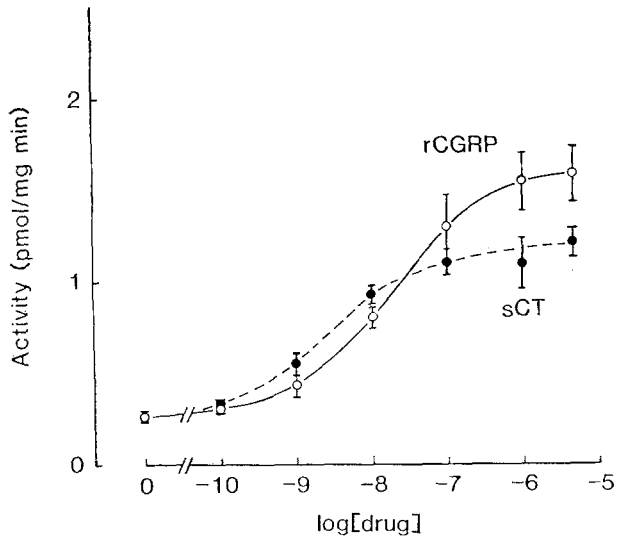

Figure 1. Effects of rCGRP and sCT on adenylate cyclase activity in rat diaphragm. The homogenate of the rat diaphragm was incubated with rCGRP (o) or sCT(•) under the conditions shown in the text. The enzyme activity was expressed as cyclic AMP formed by 1 mg (wet wt) of tissue for 1 min. Values are mean ± SEM of three separate experiments carried out in duplicate.

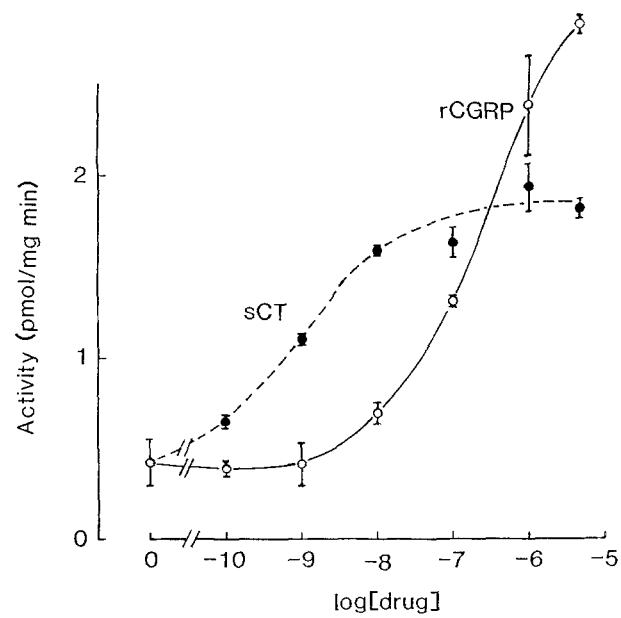

Figure 2. Effext of rCGRP and sCT on adenylate cyclase activity in rat kidney. The symbols and the experimental conditions are the same as in fig. 1.

ture (final volume of 100 µl) containing 80 mM Tris-maleate (pH 7.4), 10 mM theophylline, 100 µM GTP, 8 mM $MgSO_4$, 1 mM EGTA, 1.5 mM ATP and tissue homogenate (0.2 mg original wet wt), plus the concentration of peptides indicated. The incubation at 30 °C for 10 min was started by the addition of ATP and was stopped by the addition of 25 µl of 0.5 M HCl followed by boiling for 3 min. After centrifugation, cyclic AMP in the supernatant was measured by radioimmunoassay (Yamasa Shoyu K.K., Chiba). In this system, enzyme activity increased linearly with time and tissue concentration.

In the diaphragm, rat CGRP (rCGRP, Peninsula Laboratories, Belmont, CA) stimulated adenylate cyclase activity in a dose-dependent manner (fig. 1). The maximum activation was observed at 10^{-6} M and half-maximum stimulation was observed at 2×10^{-8} M concentration of the peptide. Salmon calcitonin (sCT, Sigma, St. Louis, MO) also stimulated adenylate cyclase activity in a dose-dependent manner with ED_{50} of 3×10^{-9} M. The maximum activation of the adenylate cyclase by rCGRP was about 50% higher than that by sCT.

Figure 2 shows the effects of rCGRP and sCT on adenylate cyclase activity in the kidney. Both peptides stimulated the enzyme activity in a dose-dependent manner. Even 5×10^{-6} M rCGRP did not elicit the maximum stimulation, so its ED_{50} value was higher than 10^{-7} M. On the other hand, the ED_{50} value for sCT in the kidney was 10^{-9} M. Accordingly, rCGRP stimulated adenylate cyclase activity with a lower ED_{50} value in the diaphragm than in the kidney, whereas sCT stimulated adenylate cyclase activity in the kidney, with a lower ED_{50} value than that observed in the diaphragm.

Our data showed that rCGRP stimulated muscle adenylate cyclase activity in a dose dependent manner. Its dose-response curve was very similar to that observed in the cyclic AMP increase in the mouse diaphragm brought about by the peptide[6]. Furthermore, in our previous paper[6] it was shown that the increase in cyclic AMP level in the diaphragm by rCGRP was strengthened in the presence of phosphodiesterase inhibitors. Therefore, the increase in cyclic AMP level in the tissue by CGRP is considered to be due to the activation of adenylate cyclase by the peptide. These observations, combined with the co-existence of CGRP with acetylcholine in the nerve ending[4], suggest an important physiological role of CGRP in the neuromuscular junction. The stimulation of adenylate cyclase by CGRP has also been reported in vascular smooth muscle[8,9].

Tschopp et al.[10] showed the presence of CGRP binding sites in the nervous tissues such as the cerebellar cortex and the spinal cord. Furthermore, Goltzman and Mitchell[7] reported the presence of discrete binding sites for rCGRP and sCT in the central nervous system as well as in peripheral tissues. They suggested that CT receptors are linked with adenylate cyclase while CGRP receptors in the spinal cord are not. However, rCGRP may cross-react with sCT receptors in the kidney, resulting in the activation of adenylate cyclase. In good agreement with their data, we observed that both sCT and rCGRP stimulated adenylate cyclase activity, and the ED_{50} value for sCT is lower than that for rCGRP in the kidney although the maximum stimulation was higher by rCGRP than by sCT. In the muscle, in comparison with the kidney, the ED_{50} for sCT is higher whereas that for rCGRP is lower, which indicates that rCGRP may stimulate adenylate cyclase by reacting with sites which are different from the CT receptors in the kidney.

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Nitroglycerin-induced desensitization of vascular smooth muscle may be mediated through cyclic GMP-disinhibition of phosphatidylinositol hydrolysis

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Summary. The purpose of this study was to investigate the hypothesis that nitroglycerin-induced desensitization of vascular smooth muscle is mediated through cyclic GMP-disinhibition of phosphatidylinositol hydrolysis. Norepinephrine-induced contraction and increased levels of inositol monophosphate, a measure of phosphatidylinositol hydrolysis, in rat aorta. Prior treatment with nitroglycerin inhibited both the norepinephrine-induced contraction and the elevated levels of inositol monophosphate to the same relative magnitude. The nitroglycerin-induced inhibition of contraction and inositol monophosphate formation were prevented in tissues desensitized with nitroglycerin. These results suggest that: 1) nitroglycerin may inhibit vascular smooth muscle contraction through cyclic GMP-inhibition of phosphatidylinositol hydrolysis and 2) desensitization to the relaxant effects of nitroglycerin may be due to disinhibition of the hydrolysis.
Key words. Vascular smooth muscle; nitroglycerin; cyclic GMP; relaxation; desensitization; phosphatidylinositol; inositol phosphates.

Tolerance to the hypotensive effects of nitroglycerin have been established clinically[1-3], and have been observed in animals treated with nitroglycerin[4-6]. The site of nitroglycerin-induced tolerance may be at the level of the vasculature, since relaxations to nitroglycerin were reduced in blood vessels removed from animals treated with nitroglycerin[4,6]. Nitroglycerin treatment of blood vessels in vitro has also been shown to inhibit their subsequent ability to relax in response to nitroglycerin[7-9]. Relaxation to the nitrovasodilators, including nitroglycerin, has been proposed to be mediated through the formation of cyclic GMP[10-13]. In support of this hypothesis, are the observations that the increased levels of cyclic GMP associated with nitroglycerin-induced relaxation are reduced in blood vessels removed from animals treated with nitroglycerin, or in vessels exposed to nitroglycerin in vitro[7,9,10,14-17]. However, the hypothesis that nitroglycerin-induced desensitization may be due to an inability to elevate cyclic GMP needs to be further evaluated, since the events which follow cyclic GMP elevation and result in relaxation, remain obscure.

There are a number of reports which have demonstrated that contraction of vascular smooth muscle is associated with increased phosphatidylinositol hydrolysis[18-20]. The increased hydrolysis of phosphatidylinositol is thought to elevate the levels of inositol trisphosphate and diacylglycerol, which may result in contraction[18,21]. Furthermore, it has been proposed that cyclic nucleotides may act as feedback inhibitors of contraction through inhibition of phosphatidylinositol hydrolysis[21]. Consistent with this hypothesis, we have recently shown that sodium nitroprusside inhibited the elevated levels of inositol monophosphate due to norepinephrine, and that this inhibitory effect was mimicked by 8-bromo cyclic GMP[22]. Thus, the purpose of the present study was to investigate whether nitroglycerin-induced tolerance in vascular smooth muscle was due to cyclic GMP-mediated disinhibition of phosphatidylinositol hydrolysis.

Materials and methods. Rats (Sprague-Dawley, male, 240–360 g) were decapitated, their thoracic aortae removed and cleaned of extraneous fatty tissue. Helical strips (approximately 2 mm × 1.5 cm) were prepared and the endothelium removed by rubbing with a scalpel[23]. Tissues were then incubated for 3 h with 8 μCi/ml ^{3}H-inositol (myo-[(2-^{3}H(N))]-inositol, 16.5 μCi/mmole, New England Nuclear) in 37 °C Krebs-Ringer bicarbonate solution which was gassed with 95% O_2–5% CO_2 and had the following composition (mM): NaCl, 118.5; KCl, 4.74; $MgSO_4$, 1.18; KH_2PO_4, 1.18; $CaCl_2$, 2.5; $NaHCO_3$, 24.9; glucose, 10.0. Strips were then mounted in organ baths and placed at 0.8 g-force resting tension which was maintained throughout the experiment. Tissues were allowed to equilibrate for 2 h prior to the addition of any drugs. Other strips were placed in flasks and desensitized by exposure for 1 h to 0.44 mM nitroglycerin (1:10 nitroglycerin:lactose from ICI Americas, Inc.) as we and others have previously described[9,15]. Control strips were exposed to 2.6 mM lactose. Lactose was added to strips unexposed to nitroglycerin since the nitroglycerin was added as a lactose powder. Tissues were then mounted in organ baths as above and washed every 15 min over the next 1 h. Strips were exposed to 10 or 100

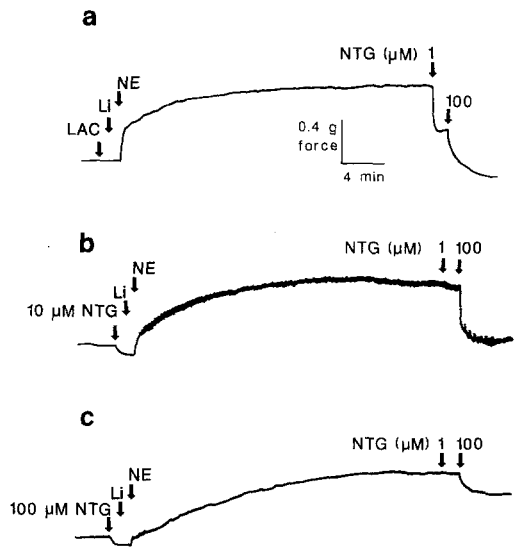

Figure 1. Effects of nitroglycerin on norepinephrine-induced contractions. Rat thoracic aortae without endothelium were exposed to 600 μM lactose (LAC; *a*), or 10 or 100 μM nitroglycerin (NTG; *b*, *c*), followed by 10 mM lithium chloride (Li) and then 0.3 μM norepinephrine (NE). Tissues were exposed to 1 and then 100 μM nitroglycerin 30 min after the addition of norepinephrine. Tracings of tension recordings are shown.