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# Impairment by lovastatin of neural relaxation of the rabbit sphincter of Oddi

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**Abstract**

We sought whether inhibition of cholesterol biosynthesis by lovastatin influenced the nitrergic relaxation response of the sphincter of Oddi. Rabbit sphincters of Oddi rings were tested for changes in isometric tension in response to field stimulation in the presence of 4 μM guanethidine and 1 μM atropine. Tissue samples were then analyzed for cAMP and cGMP content by radioimmunoassay for nitric oxide concentration by electron spin resonance and for vasoactive intestinal peptide and calcitonin gene-related peptide (CGRP) release by radioimmunoassay. Membrane $G_{s\alpha}$ protein was determined by Western blot analysis. Field stimulation relaxed the preparations with an increase in nitric oxide, cAMP and cGMP concentrations at increased calcitonin gene-related peptide and vasoactive intestinal polypeptide (VIP) release. Preparations from rabbits pre-treated with lovastatin (5 mg/kg/day intragastrically, over 5 days) contracted under the same conditions with an attenuated cGMP-increase at preserved increase in NO content and neuropeptide release. The relaxation was recaptured combining lovastatin with farnesol (1 mg/kg intravenously, twice a day for 5 days). The field stimulation-induced increase in cyclic nucleotides was also restored. Lovastatin decreased membrane $G_{s\alpha}$ protein content, which was re-normalized by farnesol. Farnesol treatment reinstates neurogenic relaxation of the sphincter of Oddi deteriorated by lovastatin possibly by normalizing G-protein coupling. © 2001 Elsevier Science B.V. All rights reserved.

*Keywords:* Sphincter of Oddi; Nitrergic relaxation; Lovastatin; Farnesol

## 1. Introduction

Non-adrenergic, non-cholinergic (NANC) relaxation of the sphincter of Oddi is a prerequisite for normal delivery of bile into the duodenum (Pauletzki et al., 1993). This mechanism is essentially nitrergic in rabbits (Lonovics et al., 1994).

We have found that farnesol supplementation improves NANC relaxation of the rabbit sphincter of Oddi deteriorated by hypercholesterolemia (Szilvassy et al., 1998). This is in accordance with results by Roullet et al. (1993, 1995) that farnesyl analogues re-normalize vascular tone deteriorated by either hypercholesterolemia or inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, a key enzyme in the mevalonate pathway independent of serum cholesterol levels. Therefore, the present work was to study if inhibition of HMG-CoA reductase by repeated administration of lovastatin decreased the NANC relaxation response in sphincters of Oddi preparations from otherwise healthy animals and as to whether this could be masked by farnesol supplementation.

A part of this work was presented in the annual meeting of the American Gastroenterological Association in San Diego, CA, 2000 (Sari et al., 2000).

## 2. Methods

### 2.1. Ethics

The experiments performed in the present work conform to European Community guiding principles for the

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care and use of laboratory animals. The experimental protocol applied has been approved by the local ethical committee of the Medical Universities of Debrecen, Szeged, and Pecs, Hungary.

### 2.2. Experimental groups

Sphincter of Oddi muscle rings were prepared from groups of adult male New-Zealand white rabbits (3000–3500 g) as follows: untreated animals 'Group 1', and from those treated with lovastatin (5 mg/kg/day intragastrically, over 5 days; MEVACOR, MSD, Rahway, NY) 'Group2', or farnesol (1 mg/kg intravenously, twice a day for 5 days; Sigma, St. Louis, MO) 'Group 3', and from those given lovastatin + farnesol over 5 days 'Group 4'. Each group consisted of 23 preparations from 23 animals, six of which were used for measurement of isometric tension; five rings entered radioimmunoassay studies for determination of baseline cAMP and cGMP, eight rings were used for determination of tissue nitric oxide (NO) and four rings per group served for Western blot analysis of the membrane-associated G protein subunit $G_{s\alpha}$. Two additional groups (nine preparations in both) were instituted to test the effect of the solvent for farnesol and the placebo for lovastatin (four preparations for isometric tension measurements, three for cyclic nucleotide- and four for NO determinations per group). To reduce the number of experimental animals, field stimulation-induced changes in tissue cyclic nucleotide concentration were determined from the same preparations as those used for isometric tension measurements (see experimental protocol).

### 2.3. Isometric tension measurement

These have been described in detail elsewhere (Pauletzki et al., 1993). Biliary sphincter of Oddi muscle rings of approximately 6 mm length from adult male New Zealand white rabbits weighing from 3000 to 3500 g were prepared. The papilla Vateri was eliminated and the ampullary part of the muscle rings of approximately 3 mm length were mounted horizontally on two small L-shaped glass hooks of which one was connected to a force transducer (SG-O2, Experimetria, Budapest, Hungary) attached to a six channel polygraph (R61 6CH, Mikromed, Budapest, Hungary) for measurement and recording of isometric tension. The experiments were carried out in an organ bath (5 ml) containing Krebs bicarbonate buffer that was maintained at 37 °C and aerated continuously with carbogen. The initial tension was set at 10 milliNewton (mN) and the rings were allowed to equilibrate over 1 h. Atropine (1 μM) and guanethidine (4 μM) were continuously present (NANC solution). Changes in isometric tension in response to two consecutive trains of impulses of electrical field stimulation (40 stimuli 50 V, 0.1 ms and 20 Hz) were then studied.

### 2.4. Determination of cyclic nucleotide content in samples from isolated rabbit sphincter of Oddi

Tissue cGMP and cAMP contents were determined by means of radioimmunoassay as described (Szilvássy et al., 1994). Briefly, the muscle rings were snap frozen (to prevent cyclic nucleotides from breakdown by phosphodiesterases) in liquid nitrogen. The samples were then homogenized in 6% trichloroacetic acid. After thawing, the samples were processed at 4 °C. Sedimentation at 15,000 $\times g$ for 10 min by means of a Janetzki K-24 centrifuge (Leipzig, Germany) was followed by extraction of supernatant with 5 ml water-saturated ether. The samples were evaporated under nitrogen, and assayed for cyclic nucleotide contents using Amersham radioimmunoassay kits (Les Ulis, France). The values were expressed as pmol/mg wet tissue weight.

Serum cholesterol level was determined as described (Szilvassy et al., 1995).

### 2.5. Membrane preparations and Western blot analysis

Sphincter of Oddi muscle rings were homogenized in ice-cold 50 mM Tris/HCl (pH 7.4 at 25 °C) containing 10 μg/ml soybean trypsin inhibitor, 5 μg/ml leupeptin, 200 μg/ml bacitracin, 2 mM EDTA and 100 μM phenylmethylsulfonyl fluoride to prevent proteolysis. The supernatant fraction resulting from centrifugation with 600 $\times g$ for 10 min was re-centrifuged at 30,000 $\times g$ for 15 min at 4 °C. The pellet was re-homogenized in fresh buffer and re-centrifuged. The final pellet was re-suspended in ice-cold assay buffer (50 mM Tris/HCl, 5 mM $MgCl_2$, pH 7.4 at 25 °C), and protein content was determined by Lowry's method using bovine serum albumin as a standard. Membrane prepararions were maintained at −80 °C for up to 2 weeks until utilized in assays. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) and Western blotting were performed using the procedure previously described by Miyamoto et al. (1992). In brief, membrane suspensions were dissolved in an equal volume of sample buffer containing 62.5 mM Tris/HCl (pH 6.8), 10% glycerol, 2% SDS, 5% mercaptoethanol and 0.0025% bromophenol blue and boiled for 5 min before application to the gel (7.5 μg protein per lane). After electrophoresis (40 mA for 100 min), the gels were soaked for 20 min in transfer buffer (25 mM Tris, 192 mM glycine, and 20% methanol). Proteins were transferred from the gel to presoaked nitrocellulose membranes at 180 mA over 90 min. The membranes were incubated for 2 h in 0.01 M Tris/HCl (pH 7.4)/0.9% NaCl containing 3% bovine serum albumin. Immunodetection was carried out by incubating the membrane with specific sheep antiserum recognizing $G_{s\alpha}$ diluted 1:2000 with the above buffer overnight at room temperature. The membranes were washed five times over 30 min. Membranes were then incubated for 2 h at room

temperature with horseradish peroxidase-conjugated goat anti-sheep IgG diluted 1:1000 with the above buffer. The antibody bound to nitrocellulose membrane was detected by the chromogenic substrate 4-chloro-1-naphtol. Immunoreactivity was also detected with the enhanced chemiluminescence Western Blot Detection System followed by exposure to Hyperfilm-enhanced chemiluminescence. Immunolabelled G proteins and the intensity of the specific bands were assayed by Soft Laser Scanning Densitometer (Biomed Instruments, USA)

### 2.6. Tissue NO determination by means of electron spin resonance

Nitric oxide content of freshly minced sphincter of Oddi tissue was measured using electron spin resonance spectroscopy after spin trapping with 55 mmol/l *N*-methyl-glucosamine-dithiocarbamate as described in details elsewhere. NO content was expressed as arbitrary units/mg tissue (Csont et al., 1998; Ferdinandy et al., 2000; Radak et al., 1999).

### 2.7. NANC neurotransmitter release studies

Calcitonin gene-related peptide (CGRP), concentrations were determined from 200 μl samples of organ fluid of the preparations by means of radioimmunoassay methods developed in our laboratories as described (Helyes et al., 1997; Nemeth et al., 1999). For radioimmunoassay determination of vasoactive intestinal polypeptide (VIP), we used commercial radioimmunoassay kits (Phoenix Pharmaceuticals, Belmont, CA, USA). Sampling was done prior to (resting values) and immediately after field stimulation (at maximum contraction/relaxation).

### 2.8. Sampling for cyclic nucleotide and NO determination

The muscle rings used for isometric tension measurements were used for radioimmunoassay studies as well, to determine field stimulation-induced changes in tissue cyclic nucleotide levels. Sampling was done so that the whole preparation exhibiting contraction/relaxation in response to field stimulation was placed in liquid nitrogen in 2 s subsequent to the maximum contractile response. For control to these series of rings served those which had not been subjected to field stimulation (resting values). The same sampling scheme was used for tissue NO measurement.

### 2.9. Drugs and chemicals

Beyond radioimmunoassay kits, all drugs and chemicals used in this study were purchased from Sigma except lovastatin and its placebo (MEVACOR, Merck-Sharp and Dohme, Hungaria Kft., Budapest, Hungary). Atropine and guanethidine were freshly dissolved in Krebs solution and added to the organ baths in 50 μl volume. Farnesol (3,7,11-trimethyl-2,2,10-dodecatrien-1-ol, mixed isomers) was diluted with 0.5 ml/kg body weight propylene glycol, therefore, propylene glycol was referred to as the solvent for farnesol.

### 2.10. Statistical analysis

The data representing changes in isometric tension and neuropeptide release expressed as means ± standard deviation (S.D.) were evaluated by means of analysis of variance followed by a modified Student's *t*-test for multiple comparisons according to Bonferroni's method. Changes in tissue cyclic nucleotide and NO contents were evaluated by means of Student's *t*-test. Changes were considered statistically significant at $P$ values smaller than 0.05.

## 3. Results

### 3.1. Isometric tension

The NANC relaxation response induced by field stimulation (20 Hz, 50 V, 0.1 ms, 40 stimuli) was converted to contraction in animals treated with lovastatin. Lovastatin–farnesol combination restored the normal NANC relaxation response (at the same field stimulation protocol). Farnesol was without effect on NANC relaxation by itself (Fig. 1). The NANC relaxation was not modified by the placebo for lovastatin or the solvent for farnesol.

### 3.2. Tissue NO content

In tissue samples of the sphincter Oddi, neither lovastatin nor farnesol or their combination influenced either baseline or post-stimulation intensity of specific spectra of NO-L-*N*-methyl-glucosamine-dithiocarbamate complex as-

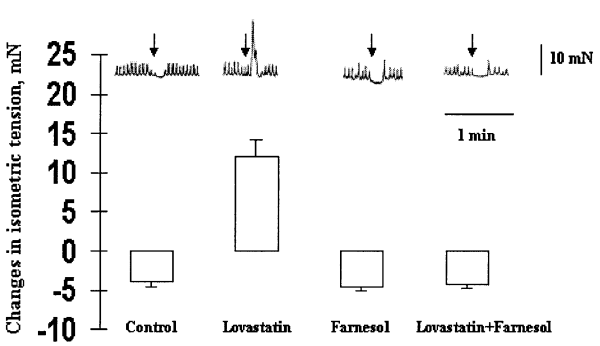


Fig. 1. Changes in isometric tension induced by electrical field stimulation (40 stimuli, 50 V, 0.1 ms and 20 Hz) in sphincter of Oddi preparations in vitro. The data are expressed as means ± S.D. obtained with six preparations in each group. Positive values indicate contraction, negative values denote relaxation. The original tracings in the upper part of the figure represent characteristic responses to field stimulation in each particular group. The arrows show commencement of field stimulation.

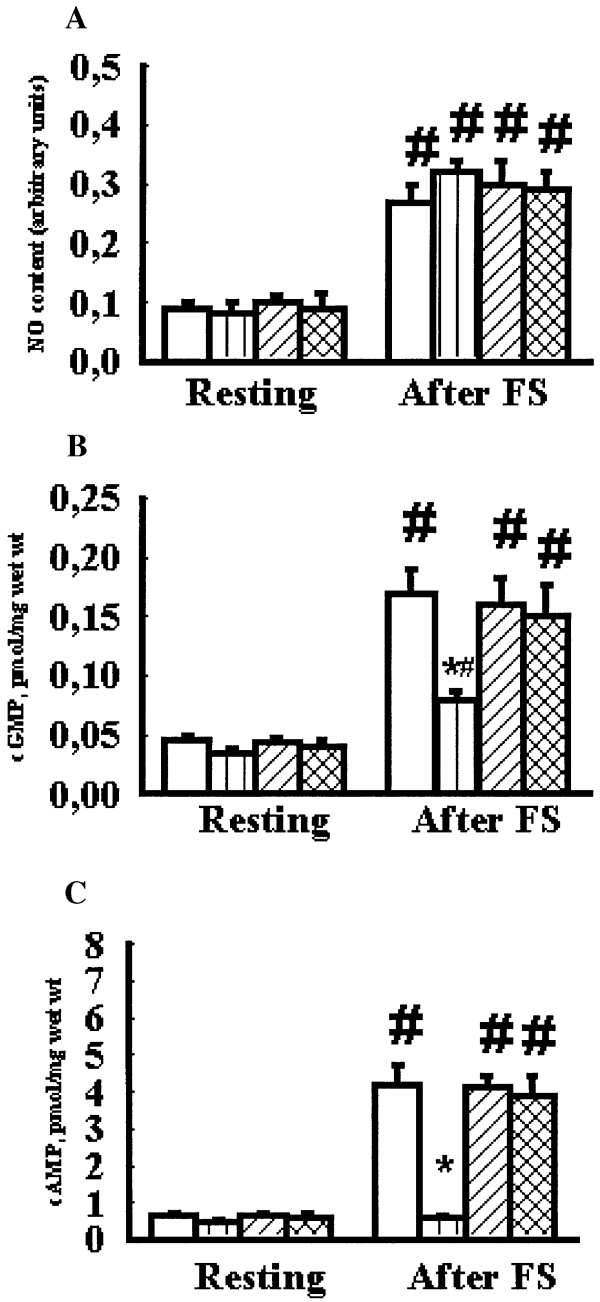


Fig. 2. Changes in tissue nitric oxide (NO, A) cGMP (B) and cAMP (C) content of sphincter of Oddi muscle rings in response to electrical field stimulation (0.1 ms, 50 V, 20 Hz, 40 stimuli). Open bars: rings from untreated animals; vertical line bars: lovastatin-treated animals; hatched bars: farnesol-treated; cross-hatched bars: lovastatin + farnesol-treated. Data are means ± S.D. obtained with six rings from six animals. *: Lovastatin vs. untreated at $P < 0.05$; #: stimulated vs. resting at $P < 0.05$.

sessed by electron spin resonance as compared to those from the untreated animals (Fig. 2A).

### 3.3. Changes in cyclic nucleotides

Field stimulation-induced NANC relaxation was accompanied by a significant increase in both cGMP and cAMP in preparations from the untreated animals. In muscle rings obtained from the lovastatin-treated group, the increase in cGMP in response to field stimulation was much lower than that seen in sphincters from the untreated rabbits. Interestingly, field stimulation failed to increase cAMP in the lovastatin-treated group. Farnesol–lovastatin combination yielded complete restoration of the increase in both cyclic nucleotides in response to field stimulation (Fig. 2B and C). Farnesol, its solvent or the placebo for lovastatin was without effect.

### 3.4. NANC neurotransmitter release studies

Field stimulation induced a significant increase in VIP and CGRP concentration in organ fluid of the preparations as determined by means of radioimmunoassay. This was not modified by any of the treatments applied (Fig. 3A and B).

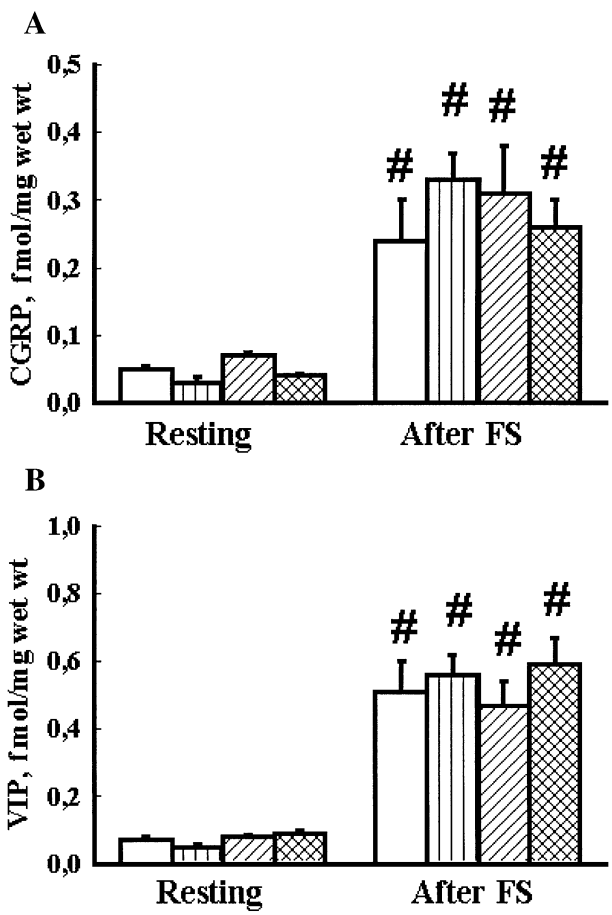


Fig. 3. Changes in calcitonin gene-related peptide (CGRP, A) and vasoactive intestinal polypeptide (VIP, B) release from isolated sphincter of Oddi muscle rings in response to electrical field stimulation (0.1 ms, 50 V, 20 Hz, 40 stimuli). Open bars: rings from untreated animals; vertical line bars: lovastatin-treated animals; hatched bars: farnesol-treated; cross-hatched bars: lovastatin + farnesol-treated. Data are means ± S.D. obtained with six rings from six animals. *: Lovastatin vs. untreated at $P < 0.05$; #: stimulated vs. resting at $P < 0.05$.

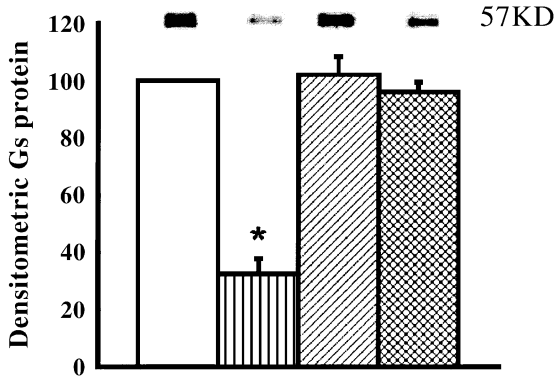


Fig. 4. Western blot analysis of $G_{s\alpha}$ subunit in rabbit sphincter of Oddi membranes. A total of 7.5 μg protein was loaded per lane. A representative gel obtained with preparations from untreated (open bars), lovastatin-treated (vertical line bars), farnesol-treated (hatched bars) and lovastatin + farnesol-treated (cross-hatched bars) animals is shown in the inset at the top of each corresponding bar. Densitometric results are expressed as a percent of density seen with preparations from untreated rabbits. Data are means ± S.D. obtained with four preparations from four animals. *: Treated vs. untreated at $P < 0.05$.

### *3.5. Membrane composition of $G_{s\alpha}$ protein in the sphincter of Oddi*

To estimate the effect of HMG-CoA inhibition on membrane particulation of G proteins in the sphincter of Oddi, quantification of $G_{s\alpha}$ protein, a representative of the membrane bound G protein complex, was done by immunodetection using specific $G_{s\alpha}$ antibody. The $G_{s\alpha}$ antiserum recognized a 57-kDa band, the density of which substantially decreased in membrane preparations from sphincter from animals treated with lovastatin (Fig. 4). Farnesol supplementation revealed re-normalization of $G_{s\alpha}$ density in muscle from the lovastatin-treated group. Farnesol was without effect (Fig. 4).

### *3.6. Serum cholesterol level*

Serum total cholesterol level of $2.1 \pm 0.3$ mmol/l decreased by $1.6 \pm 0.2$ and $1.5 \pm 0.1$ mmol/l ($P < 0.01$ for both) by lovastatin and lovastatin + farnesol, respectively. Farnesol, the solvent for farnesol or the placebo for lovastatin were without effect.

## 4. Discussion

These results show that a 5-day treatment with lovastatin, a prototype of HMG-CoA inhibitors impairs neurogenic relaxation of the rabbit sphincter of Oddi, a mechanism shown to be nitrergic in nature (Lonovics et al., 1994). In fact, the relaxation response was converted to contraction in preparations from the lovastatin-treated animals similar to that seen after NO synthase inhibition (Lonovics et al., 1994). However, the present electron spin resonance studies reveal that lovastatin is without effect on NO synthesis since neither baseline nor post-stimulation tissue NO levels differed from those observed in muscle rings from untreated animals. Interestingly, the cGMP-increase in response to field stimulation was significantly lower in preparations from the lovastatin-treated animals than that in the untreated group, with loss of the stimulation-induced cAMP-increase after 'lovastatin'. Treatment with farnesol attained a complete restoration of both neural relaxation and the cyclic nucleotide responses deteriorated by lovastatin.

It is widely accepted that NO, through cGMP synthesis, induces a sequence of protein phosphorylation that leads to smooth muscle relaxation (Moncada et al., 1991). The present results similar to that previously observed show that nitrergic relaxation of the rabbit sphincter of Oddi is accompanied by an increase in both cGMP and cAMP concentration (Szilvássy et al., 1997a). Theoretically, an increase in tissue cAMP concentration secondary to cGMP-increase might result from an inhibition of cAMP metabolism through inhibition of the enzyme type III (cGMP-inhibited cyclic nucleotide phosphodiesterase: PDE3) phosphodiesterase (Walter, 1984; Manganiello et al., 1995). However, the simultaneous increase in tissue cGMP and cAMP concentration in response to field stimulation can better be attributed to the co-release of NO and VIP (Grider and Jin, 1993) the latter of which is known to cause activation of specific membrane receptors coupled to a G protein complex for stimulation of adenylate cyclase and to increase cAMP (Altiere and Diamond, 1984; Chakder and Rattan, 1993). Notwithstanding, cAMP may increase in response to field stimulation due to adenylate cyclase stimulation by neurotransmitters released from intrinsic or sensory nerve terminals of the sphincter of Oddi other than VIP such as CGRP (Sand et al., 1993; Rasmussen et al., 1997). Moreover, in addition to its smooth muscle relaxing effect, NO has been proposed to stimulate the release of VIP (and perhaps other cAMP elevating agents) from enteric nerve terminals through presynaptic mechanisms (Allescher et al., 1996). VIP or CGRP, once released, facilitates further NO synthesis/release through cAMP-dependent pathways, thus, an interplay between NO and these NANC peptides underlie the increase in cGMP and cAMP in the sphincter of Oddi. Our present results are in good agreement with these observations, since the field stimulation-induced NANC relaxation was accompanied by a significant increase in NO, CGRP and VIP with an ensuing increase in both cAMP and cGMP.

The major original finding of the present work is that a 5-day treatment with the HMG-CoA reductase inhibitor lovastatin similar to dietary hypercholesterolemia (Szilvassy et al., 1996) abolished the NANC relaxation phenomenon at least under our experimental conditions. In the vasculature, functional defects have long been indentified in endothelial cells in hypercholesterolemia underlain by a deficiency in the release/some of the effects of NO both of which requiring the integrity of several G protein

effector systems (Flavahan, 1992). To fulfil their biological function, G proteins must undergo a post-translation modification with farnesyl or geranylgeranyl moieties that enable them to associate with the membrane. The availability of these moieties, however, is reduced by both dietary hypercholesterolemia (Goldstein and Brown, 1990) and as a result of HMG-CoA reductase inhibition. We therefore considered possible that a 5-day treatment with the HMG-CoA reductase inhibitor lovastatin influenced nitrergic relaxation in the gastrointestinal tract in a similar way, resulting in a deficiency of both the release and effect of NO. The present results only partially support this assumption since the release of neither NO nor that of the two relaxant peptides was attenuated by lovastatin treatment. However, possibly due to its G protein dependence, no cAMP-increase was seen in sphincters from the lovastatin-treated group in response to field stimulation, whereas the G protein-independent cGMP formation was only partially impaired. The relative deficiency in cGMP formation after lovastatin may reflect the possible cAMP-dependent releasing effect of VIP and CGRP on NO, a mechanism confirmed in the vasculature (Sano et al., 2000). This latter mechanism, however, is not evident from our results, possibly due to the semi-quantitative nature of the electron spin resonance technique used for tissue NO determination, since approximately the same NO levels were seen with or without lovastatin. Furthermore, the smooth muscle relaxing effect of cGMP has been shown to involve a G protein-dependent activation of potassium channels that contributes to G protein-independent relaxation pathways (Ohno et al., 1993) (Fig. 5). Thus, the effect of NANC contractile mechanisms was unopposed during field stimulation in preparations from the lovastatin-treated animals in part due to a deficiency in the interplay between cAMP and cGMP elevating agents supplemented with partial loss of cGMP effects. After combined treatment with lovastatin and farnesol, the normal NANC relaxation response was regained with an increase in tissue cGMP content in response to field stimulation.

These results seem to support the assumption that a deficiency in the synthesis of non-cholesterol mevalonate products induced by either experimental hypercholesterolemia or pharmacological inhibition of HMG-CoA reductase, the key enzyme of cholesterol biosynthesis impairs NANC relaxation of the sphincter of Oddi. Farnesol supplementation, however, restored the relaxation response in both cases (Szilvassy et al., 1998).

Recent studies have suggested that non-cholesterol mevalonate products are implicated in the control of vascular tone and blood pressure (Roullet et al., 1995, 1996). Since NANC relaxation is a pre-requisite for normal delivery of bile into the duodenum, a mechanism vulnerable to lovastatin, it is strongly suggested that non-sterol mevalonate-derived metabolites significantly contribute to the control of extrahepatic biliary tract motility as well (Szilvassy et al., 1998). This is supported by the fact that farnesol, the natural dephosphorylated form of farnesyl pyrophosphate that participates in protein farnesylation, recaptures the normal NANC relaxation function deteriorated by either hypercholesterolemia (Szilvassy et al., 1998) or HMG-CoA reductase inhibition. In certain clinical cases, however, a 1-month treatment with a low lovastatin dose (20 mg/kg in the evening), alleviated post-prandial right upper quadrant pain and improved the responsiveness to amylnitrite to enhance transpapillary bile flow as confirmed by results from hepatobiliary scintigraphy (Szilvássy

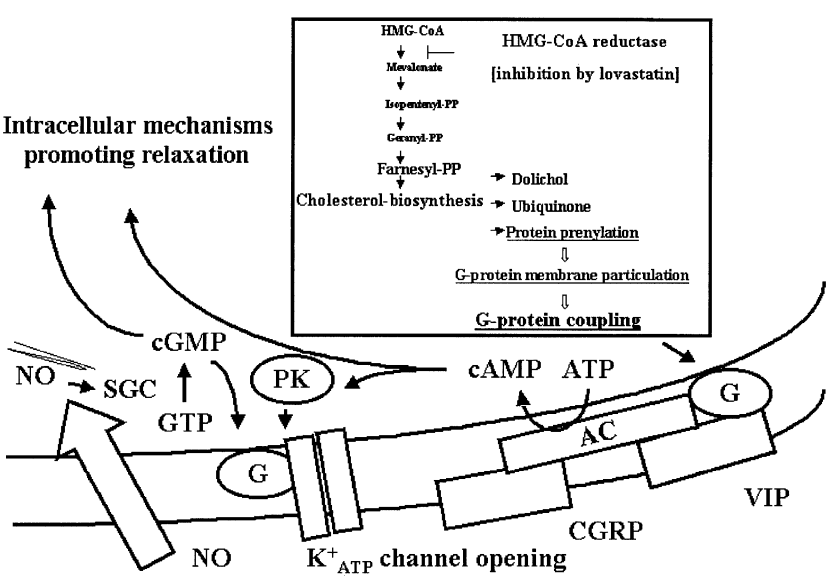


Fig. 5. Schematic diagram of the link between isoprenoid biosynthesis and mechanisms underlying NANC relaxation of the sphincter of Oddi. Inhibition of HMG-CoA by lovastatin decreases formation of non-cholesterol mevalonate products such geranyl-PP and farnesyl-PP important in protein prenylation, a process that plays a key role of membrane particulation of G proteins. Thus, inhibition by lovastatin of HMG-CoA reductase results in a deficiency in G protein-dependent signal transduction pathways such as cAMP formation and activation of potassium channels.

et al., 1997b). The virtual contradiction may at least in part be explained by the difference in the degree of HMG-CoA reductase inhibition in different tissues in different species and that lovastatin may mask the effect of hypercholesterolemia that deteriorates the relaxation function of the sphincter of Oddi by itself.

Beyond providing further evidence that non-sterol mevalonate products participate in widespread physiological regulatory mechanisms including sphincter of Oddi function, the results call attention to the non-lipid lowering effect of HMG-CoA reductase inhibitors which should be taken into account especially with long-term use of these drugs.

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## References

Allescher, H.D., Kurjak, M., Huber, A., Trudrung, P., Schusdziarra, V., 1996. Regulation of VIP release from rat enteric nerve terminals: evidence for a stimulatory effect of NO. Am. J. Physiol. 271, G568–G574.

Altiere, R.J., Diamond, L., 1984. Comparison of vasoactive intestinal peptide and isoproterenol relaxant effect in isolated cat airways. J. Appl. Physiol.: Respir., Environ. Exercise Physiol. 56, 986–992.

Chakder, S., Rattan, S., 1993. Involvement of cAMP and cGMP in relaxation of internal anal sphincter by neural stimulation, VIP, and NO. Am. J. Physiol. 264, G6702–G6707.

Csont, T., Pali, T., Szilvassy, Z., Ferdinandy, P., 1998. Lack of correlation between myocardial nitric oxide and cyclic guanosine monophosphate content in both nitrate tolerant and non-tolerant rats. Biochem. Pharmacol. 56, 1139–1144.

Ferdinandy, P., Daniel, H., Ambrus, I., Rothery, R.A., Schulz, R., 2000. Peroxynitrite is a major contributor to cytokine-induced myocardial contractile failure in rat hearts. Circ. Res. 87, 241–247.

Flavahan, N.A., 1992. Atherosclerosis or lipoprotein-induced endothelial dysfunction. Potential mechanisms underlying reduction in EDRF/nitric oxide activity. Circulation 85, 1927–1938.

Goldstein, J.L., Brown, M.S., 1990. Regulation of mevalonate pathway. Nature 343, 425–430.

Grider, J.R., Jin, J.G., 1993. Vasoactive intestinal polypeptide and L-citrulline production from isolated ganglia of the myenteric plexus: evidence for regulation of vasoactive intestinal peptide release by nitric oxide. Neuroscience 54, 521–526.

Helyes, Z., Nemeth, J., Pinter, E., Szolcsanyi, J., 1997. Inhibition by nociceptin of neurogenic inflammation and the release of SP and CGRP from sensory nerve terminals. Br. J. Pharmacol. 121, 613–615.

Lonovics, J., Jakab, I., Szilvassy, J., Szilvassy, Z., 1994. Regional differences in nitric oxide-mediated relaxation of the rabbit sphincter of Oddi. Eur. J. Pharmacol. 255, 117–122.

Manganiello, V.C., Taira, M., Degerman, E., Belfrage, P., 1995. Type III cGMP inhibited cyclic nucleotide phosphodiesterases (PDE3 gene family). Cell. Signalling 7, 445–455.

Miyamoto, A., Villalobos-Molina, R., Kowatch, M.A., Roth, G.S., 1992. Altered coupling of $\alpha_1$-adrenergic receptor–G protein in rat parotid during aging. Am. J. Physiol. 262, C1181–C1188.

Moncada, S., Palmer, M.J., Higgs, E.A., 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol. Rev. 43, 109–142.

Nemeth, J., Szilvassy, Z., Than, M., Oroszi, G., Sari, R., Szolcsanyi, J., 1999. Decreased neuropeptide release from trachea of rats with streptozotocin-induced diabetes. Eur. J. Pharmacol. 369, 221–224.

Ohno, M., Gibbons, G.H., Dzau, V.J., Cooke, J.P., 1993. Shear stress elevates endothelial cGMP. Role of a potassium channel and G protein coupling. Circulation 88, 193–197.

Pauletzki, J.G., Sharkey, K.A., Davison, J.S., Bomzon, A., Shaffer, E.A., 1993. Involvement of L-arginine-nitric oxide pathways in the neural relaxation of the sphincter of Oddi. Eur. J. Pharmacol. 232, 263–270.

Radak, Z., Pucsok, J., Csont, T., Ferdinandy, P., 1999. Muscle soreness-induced reduction in force generation is accompanied by increased nitric oxide content and DNA damage in human skeletal muscle. Free Radical Biol. Med. 26, 1059–1063.

Rasmussen, T.N., Harling, H., Rehfeld, J.F., Holst, J.J., 1997. Calcitonin gene-related peptide (CGRP), a potent regulator of biliary flow. Neurogastroenterol. Motil. 9, 215–220.

Roullet, J.B., Xue, H., Pappu, A.S., Roullet, C.M., Holcomb, S., McCarron, D.A., 1993. Mevalonate availability and cardiovascular functions. Proc. Natl. Acad. Sci. U. S. A. 90, 11728–11732.

Roullet, J.B., Xue, H., Roullet, C.M., Fletcher, W.S., Cipolla, M.J., Harker, C.T., McCarron, D.A., 1995. Mevalonate availability controls human and rat resistance vessel function. J. Clin. Invest. 96, 239–244.

Roullet, J.B., Xue, H., Chapman, J., McDougal, P., Roullet, C.M., McCarron, D.A., 1996. Farnesyl analogues inhibit vasoconstriction in animal and human arteries. J. Clin. Invest. 97, 2384–3290.

Sand, J., Tainio, H., Nordback, I., 1993. Neuropeptides in pig sphincter of Oddi, bile duct, gallbladder, and duodenum. Dig. Dis. Sci. 38, 694–700.

Sano, H., Miyata, A., Sato, K., Kaiya, H., Matsuo, H., Kangawa, K., 2000. PACAP augments nitric oxide synthesis in rat vascular smooth muscle cells stimulated with IL-1 alpha. Ann. N. Y. Acad. Sci. 921, 415–419.

Sari, R., Szilvassy, Z., Nagy, I., Jakab, I., Nemeth, J., Lonovics, J., 2000. The role of cholesterol and non-cholesterol mevalonate products in the nitrergic relaxation of the sphincter of Oddi. Gastroenterology 118, A202.

Szilvássy, Z., Ferdinandy, P., Bor, P., Jakab, I., Lonovics, J., Koltai, M., 1994. Ventricular overdrive pacing-induced anti-ischemic effect: a conscious rabbit model of preconditioning. Am. J. Physiol. 266, H2033–H2041.

Szilvassy, Z., Ferdinandy, P., Szilvassy, J., Nagy, I., Karcsu, S., Lonovics, J., Dux, L., Koltai, M., 1995. Loss of preconditioning in atherosclerotic rabbits: the role of hypercholesterolaemia. J. Mol. Cell. Cardiol. 27, 2559–2569.

Szilvassy, Z., Nagy, I., Szilvassy, J., Jakab, I., Csati, S., Lonovics, J., 1996. Impaired nitrergic relaxation of the sphincter of Oddi of hyperlipidaemic rabbits. Eur. J. Pharmacol. 301, R17–R18.

Szilvássy, Z., Lonovics, J., Jakab, I., Nagy, I., 1997a. Glibenclamide sensitivity of neural relaxation of the rabbit sphincter of Oddi. Pharmacol. Res. 36, 129–133.

Szilvássy, Z., Nagy, I., Madacsy, L., Hajnal, F., Takacs, T., Velosy, B., Lonovics, J., 1997b. Beneficial effect of lovastatin on sphincter of Oddi dysfunction in hypercholesterolaemia and hypertriglyceridaemia. Am. J. Gastroenterol. 92, 900–902.

Szilvassy, Z., Sari, J., Nemeth, J., Nagy, I., Lonovics, J., 1998. Improvement of nitrergic relaxation by farnesol of the sphincter of Oddi from hypercholesterolaemic rabbits. Eur. J. Pharmacol. 353, 75–78.

Walter, U., 1984. Cyclic GMP-regulated enzymes and their physiological functions. In: Greengard, P., Robinson, A.G. (Eds.), Advances in Cyclic Nucleotide Research and Protein Phosphorylation Research. Raven Press, New York, NY, pp. 249–258.