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Short communication

Improvement of nitrergic relaxation by farnesol of the sphincter of Oddi from hypercholesterolaemic rabbits

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Abstract

Field stimulation relaxed the rabbit sphincter of Oddi muscle rings after incubation with atropine (1 μM) and guanethidine (4 μM) with a threefold increase in tissue cyclic cGMP content, a response previously shown to be essentially nitrergic. Preparations from hypercholesterolaemic rabbits (1.5% dietary cholesterol load over 8 weeks increasing serum total cholesterol from pre-diet 1.4 ± 0.3 to 22.6 ± 3.8 mmol/l) exhibited contractions with no change in cyclic GMP content under the same conditions. The nitrergic relaxation was recaptured with a twofold increase in tissue cyclic GMP content in preparations from hypercholesterolaemic rabbits undergone a treatment with 30 μM/kg farnesol i.v. twice a day over the last 3 days of the dietary period. We conclude that farnesol treatment restores nitrergic relaxation of the sphincter of Oddi in hypercholesterolaemia. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Sphincter of Oddi; Hypercholesterolaemia; Nitrergic relaxation; Farnesol

1. Introduction

The integrity of the relaxation function of the sphincter of Oddi is a prerequisite for normal delivery of bile into the duodenum. Sphincter of Oddi relaxation is mainly executed by non-adrenergic, non-cholinergic (NANC) nerves that are essentially nitrergic in several species including guinea pigs (Pauletzki et al., 1993) and rabbits (Lonovics et al., 1994).

We have shown that hypercholesterolaemia/atherosclerosis impairs relaxation function of the sphincter of Oddi in both experimental animals and clinical patients (Szilvassy et al., 1996, 1997b). Moreover, the relaxant effect of exogenous non-enzymatic NO donors on the sphincter of Oddi has also been found impaired in hypercholesterolaemia (Szilvassy et al., 1997b). Since dietary hypercholesterolaemia may impair both the release and effect of NO through a deficiency in G protein coupling at least in part due to a reduced synthesis of farnesyl or geranylgeranyl products (Flavahan, 1992), the present work was concerned with the possibility that farnesol supplementation could elicit a partial recovery of nitrergic relaxation of the sphincter of Oddi preparations from hypercholesterolaemic rabbits 'in vitro'.

2. Methods

The experiments performed in the present work conform to European Community guiding principles for the care and use of laboratory animals. The experimental protocol applied has been approved by the local ethical committee of the Albert Szent-Gyorgyi Medical University Szeged, Hungary.

2.1. Experimental groups

The study was carried out with four experimental groups (with 11 animals in each). Group 1: normal animals treated

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with the solvent for farnesol two times a day over 3 days; Group 2: normal animals treated with farnesol (30 μmol/kg b.wt.) two times a day over 3 days; Group 3: hyperlipidaemic rabbits (1.5% dietary cholesterol load over 8 weeks) treated with the solvent for farnesol; Group 4: hyperlipidaemic rabbits treated with farnesol. Each group was divided into two subgroups: six preparations from six animals were used for isometric tension measurements, whereas five muscle rings from five rabbits were used for determination of cyclic GMP by means of radioimmunoassay.

2.2. Isometric tension measurement

These have been described in detail elsewhere (Lonovics et al., 1994). Biliary sphincter of Oddi muscle rings of approximately 6 mm length from adult male New Zealand white rabbits weighing from 3500–4000 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 which 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.

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

Tissue cyclic GMP content was determined by means of radioimmunoassay as described (Szilvassy et al., 1994, 1997a). Briefly, the muscle rings were snap frozen (to prevent cyclic GMP from breakdown by phosphodiesterases) in liquid nitrogen. The samples were then homogenized in 6% trichloroacetic acid of 10 times higher quantity than sample weight in a mortar previously kept at −70°C. 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 in a Vortex extractor (MTA, Kutesz, type 5191, Budapest, Hungary) over 5 min. The ether fraction was eliminated, and the extraction was then repeated five times. The samples were evaporated under nitrogen, and assayed for cyclic GMP 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).

2.4. Drugs and chemicals

All drugs and chemicals used in this study were purchased from Sigma (St. Louis, MO, USA). 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 b.wt. propylene glycol, therefore, propylene glycol was referred to as the solvent for farnesol.

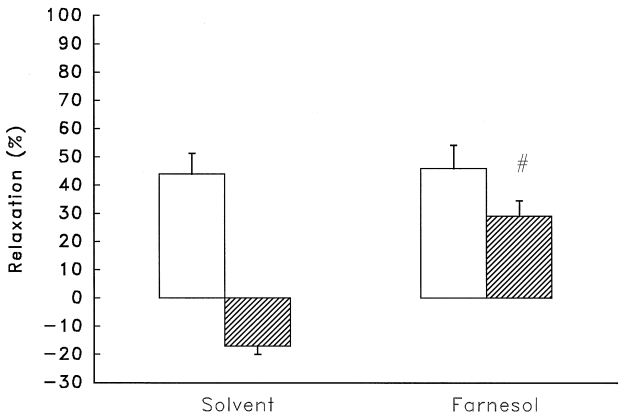

Fig. 1. Non-adrenergic, non-cholinergic relaxation of the sphincter of Oddi muscle rings from normal and hypercholesterolaemic rabbits. The effect of farnesol. Data are means ± S.D. obtained with six rings from six animals. Relaxation is expressed as a percentage decrease of the initial tension. Open bars: rings from normal animals; hatched bars: rings from hypercholesterolaemic animals. #: atherosclerotic vs. normal at $P < 0.05$.

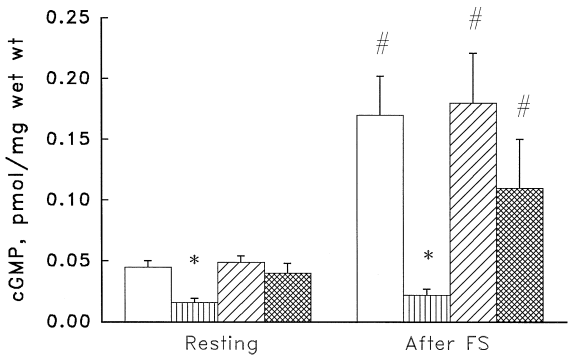

Fig. 2. Changes in cyclic GMP (cGMP) content of sphincter of Oddi muscle rings in response to electrical field stimulation (0.1 ms, 50 V, 20 Hz, 40 stimuli). Open bars: rings from solvent-treated normal animals; vertical line bars: solvent-treated atherosclerotic; hatched bars: farnesol-treated normal; cross-hatched bars: atherosclerotic, farnesol-treated. Data are means ± S.D. obtained with five rings from five animals. *: atherosclerotic vs. normal at $P < 0.05$; #: stimulated vs. resting at $P < 0.05$.

2.5. Statistical analysis

The data representing changes in isometric tension expressed as means ± standard deviation (S.D.) were evaluated by means of analysis of variance (ANOVA) followed by a modified Student's *t*-test for multiple comparisons according to Bonferroni's method. Changes in tissue cyclic nucleotide contents were evaluated by means of Student's *t*-test. Changes were considered statistically significant at *P*-values smaller than 0.05.

3. Results

The cholesterol-enriched diet increased serum cholesterol to 22.6 ± 3.8 vs. pre-diet 1.4 ± 0.3 mmol/l. In rabbits fed normal chow, serum cholesterol did not change during the same period and farnesol was without effect on serum cholesterol level.

Field stimulation-induced monophasic NANC relaxation in sphincter of Oddi muscle rings from normal rabbits treated with the solvent for farnesol (Fig. 1). Treatment with farnesol (30 μmol/kg b.wt.) did not modify this response (not shown). Preparations from the solvent-treated hypercholesterolaemic rabbits responded with NANC contractions to field stimulation (Fig. 1). However, an apparent NANC relaxation response was seen in preparations from Group 4 (Fig. 1).

Field stimulation increased cyclic GMP concentration in 'Group 1' muscle rings incubated in 'NANC solution'. Group 2 preparations exhibited the same resting and post-stimulation cyclic GMP values. Hypercholesterolaemia completely blocked the post-stimulation cyclic GMP-increase. Baseline cyclic GMP content was also decreased by hypercholesterolaemia. Post-stimulation cyclic GMP values in sphincters from hypercholesterolaemic rabbits treated with farnesol reached approximately 60% of those measured in preparations from normal animals (Fig. 2).

4. Discussion

These data confirm that NANC relaxation of the rabbit sphincter of Oddi is seriously impaired in experimental hypercholesterolaemia (Szilvassy et al., 1996). However, this report is the first to describe farnesol, a major polyprenyl product of cholesterol biosynthesis to improve relaxation function of the sphincter of Oddi deteriorated by hypercholesterolaemia.

With regard to the primary mechanism involved in NANC relaxation of the sphincter of Oddi, most evidence favours a role for NO and vasoactive intestinal polypeptide (VIP) (Pauletzki et al., 1993). We found that using the same field stimulation parameters, neural relaxation of this sphincter was completely blocked by L-NAME, an inhibitor of NO-synthase. The inhibitory effect of L-NAME was reversed by concurrent incubation with L-arginine but not D-arginine indicating the response to be essentially nitrergic in nature at this rate of stimulation (Lonovics et al., 1994; Szilvassy et al., 1996). It is widely accepted that NO evokes smooth muscle relaxation through formation of cyclic GMP within muscle cells (Moncada et al., 1991). Nevertheless, NO has been shown to stimulate the release of VIP from enteric nerve terminals, an effect to enhance or contribute to its 'per se' relaxant effect (Allesher et al., 1996).

In the vasculature, functional defects have long been identified in endothelial cells in hypercholesterolaemia underlain by a deficiency in the release and effect of NO both of which requiring the integrity of several G protein effector systems (for review, see Flavahan, 1992). To fulfil their biological function, G proteins must undergo a post-

translation modification with farnesyl or geranyl moieties that enable them to associate with the membrane. The availability of these moieties, however, is reduced in dietary hypercholesterolaemia (Goldstein and Brown, 1990). It is possible that dietary hypercholesterolaemia influences nitrergic relaxation in the gastrointestinal tract in a similar way, resulting in a deficiency of both the release and effect of NO reflected in a field stimulation-induced slight contractile response seen with sphincters from hypercholesterolaemic animals, possibly underlain by NANC contractile mechanisms awaken since the lack of opposing effects. The present results might support this assumption since NANC relaxation of the sphincter of Oddi preparations became nearly normal after a 3-day treatment with farnesol with an increase in tissue cyclic GMP content in response to field stimulation in rings from hypercholesterolaemic rabbits. Thus, in spite of the lack of any attenuation of hypercholesterolaemia itself, farnesol supplementation confers an improvement on NANC relaxation function of rabbit sphincter of Oddi 'in vitro'. Similarly, farnesyl analogues have been found to re-normalize vascular tone deteriorated by either hypercholesterolaemia or inhibition of 3-hydroxy-3-methyl-glutarylcoenzyme A (HMG-CoA) reductase, a key enzyme in the mevalonate pathway independent of plasma cholesterol levels (Roullet et al., 1993, 1995). Moreover, the rapid pacing-induced ischaemic preconditioning phenomenon of the isolated working rat heart, another NO-dependent process (Ferdinandy et al., 1997) has been found to be similarly deterioted by both experimental hypercholesterolaemia and HMG-CoA reductase inhibitor treatment (Ferdinandy et al., 1998). Of course, based on the present results, it is not possible to define the precise mechanism of action of farnesol to provide a beneficial influence on nitrergic relaxation of the sphincter of Oddi from hypercholesterolaemic animals. Nevertheless, the parallelism of an improved nitrergic relaxation function and the increased ability of 'hypercholesterolaemic' sphincters to produce cyclic GMP after treatment with farnesol suggests the importance of the availability of non-cholesterol mevalonate products in the control of extrahepatic biliary tract motility through the NO-cyclic GMP system in metabolic diseases such as hypercholesterolaemia and atherosclerosis either with or without involvement of the G-protein system.

Whatever the precise mechanism is, the results call attention to the potential clinical use of these non-cholesterol mevalonate products in the treatment of hypercholesterolaemia.

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