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Impaired nitrergic relaxation of the sphincter of Oddi of hyperlipidaemic rabbits

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Abstract

Field stimulation relaxed the sphincter of Oddi muscle rings of the rabbit after incubation with phentolamine, oxprenolol and atropine (all 1 μM). The relaxation was blocked by N^G-nitro-L-arginine methyl ester (30 μM) and was reversed by 3 mM L-arginine but not D-arginine. Sphincter of Oddi preparations from hypercholesterolaemic rabbits exhibited contractions under the same conditions. We conclude that nitrergic relaxation is impaired in the sphincter of Oddi from hypercholesterolaemic rabbits.

Keywords: Nitric oxide (NO); Hypercholesterolemia; Sphincter of Oddi

The relaxation function of the spincter of Oddi is mainly regulated by non-adrenergic non-cholinergic ('NANC') nerves that are essentially nitrergic in rabbits (Lonovics et al., 1994). It is suggested that hypercholesterolaemia attenuates either the release or the effect of nitric oxide (NO) in blood vessels (Verbeuren et al., 1986). Nevertheless, alterations in NANC relaxation of the sphincter of Oddi in hypercholesterolaemia have not been investigated. The present work aimed to study whether nitrergic relaxation of the sphincter of Oddi was altered in hypercholesterolaemic rabbits.

Groups of male New Zealand rabbits (3500–3700 g) were fed commercial rabbit chow ($n = 6$) and chow enriched with 1.5% cholesterol ($n = 6$) over 8 weeks. The serum cholesterol level was determined before and after the 8-week period as described (Szilvassy et al., 1995). The animals were then stunned and exsanguinated. Ampullary parts of sphincter of Oddi muscle rings (3 mm) were suspended on glass hooks in an organ bath (5 ml) containing Krebs bicarbonate buffer (mM: NaCl 118.1, KCl 4.7, $MgSO_4$ 1.0, KH_2PO_4 1.0, $CaCl_2$ .5, $NaHCO_3$ 25.0, glucose 11.1) maintained at 37°C and aerated continuously with 95% O_2 and 5% CO_2. The glass hooks were connected to a force transducer for measurement of isometric tension as described (Lonovics et al., 1994). The initial tension was set at 20 mN and the rings were allowed to equilibrate over 1 h. Then electrical field stimulation was applied as described (Lonovics et al., 1994). Contractile responses to 10 stimuli (50 V, 0.1 ms and 20 Hz) were studied. The rings were then preincubated with phentolamine, oxprenolol and atropine (all 1 μM) for 20 min. This was followed by three consecutive 20-min incubations with N^G-nitro-L-arginine methyl ester, 3 mM D-arginine or L-arginine. Field stimulation was applied after each period. Following washout, the stimulation protocol was repeated to test whether 'predrug' contractile responses could be reproduced. All compounds (Sigma, St. Louis, MO) dissolved in Krebs solution were added to the organ bath in a 50-μl volume. The data expressed as means $\pm$ S.D. were analysed with a one-way analysis of variance followed by the Bonferroni t-test. Changes were considered significant at $P < 0.05$.

A cholesterol-enriched diet increased serum cholesterol to 26.1 $\pm$ 4.0 vs. pre-diet 1.8 $\pm$ 0.29 mmol/l ($P < 0.001$). In rabbits fed normal chow, serum cholesterol did not change during the same period.

Field stimulation induced NANC relaxations in normal sphincters, an effect that was reversed to contractions after incubation with N^G-nitro-L-arginine methyl ester. L-Arginine but not D-arginine reversed this inhibitory effect (Fig. 1). The preparations from hypercholesterolaemic rabbits responded with contractions to field stimulation after incubation with NANC solution. Successive incubations with either arginine analogue failed to influence these contractions.

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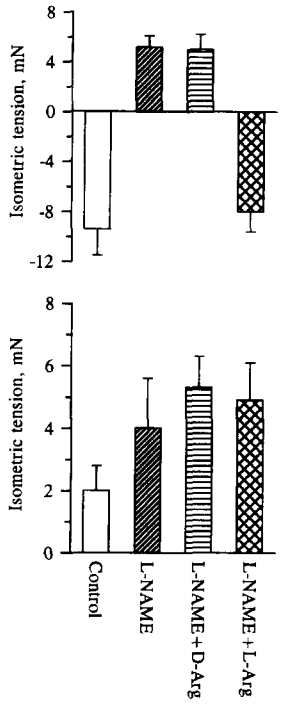

Fig. 1. Effect of electrical field stimulation on motility of isolated sphincter of Oddi from normal (upper panel) and hypercholesterolaemic (lower panel) rabbits. Maximum contraction/relaxation responses to 10 stimuli (50 V, 0.1 ms, 20 Hz) are expressed as mN (means ± S.D. obtained with 6 preparations) in the control (responses in NANC solution, i.e. after incubation with phentolamine, oxprenolol and atropine (1 μM for each) and after three consecutive incubations with 30 μM N^G-nitro-L-arginine methyl ester (L-NAME), 3 mM D-arginine and L-arginine.

These data confirm that NANC relaxation of the normal sphincter of Oddi of the rabbit is essentialy nitrergic (Lonovics et al., 1994). However, this report is the first to describe NANC relaxation of this sphincter as impaired by hypercholesterolaemia. In the vasculature, functional defects have been identified in endothelial cells in hypercholesterolaemia (Verbeuren et al., 1986). We also have found that 8-week exposure to a 1.5% cholesterol-enriched diet results in impairment of endothelium-dependent vasodilation (Szilvassy et al., 1995). Previously, reduced endothelium-dependent vasorelaxation due to hypercholesterolaemia has been proposed to have as basis a reduced formation and/or release of endothelium-derived relaxing factor (EDRF) identified as NO. Nevertheless, the diminished nitrergic response may result from more rapid inactivation of the NO released. Ohara et al. (1993) have shown an increased superoxide anion production in hypercholesterolaemia and an improvement of EDRF-dependent relaxation by superoxide dismutase; however, inactivation of NO by low-density lipoproteins may also be involved (Jacobs et al., 1990). It is also possible that NO is less active on gastrointestinal smooth muscle cells because of alterations in the signal transduction pathway within the muscle cells. Studies with exogenous NO donors on sphincter of Oddi motility would clarify this point since, if the latter were true, relaxation responses to nitroglycerin or sodium nitroprusside should also be disturbed in hypercholesterolaemia.

In summary, the study demonstrated the impairment of the endogenous relaxation mechanism of the sphinter of Oddi from hypercholesterolaemic animals, indicating a possible role of hypercholesterolaemia in papillary dysfunction-related diseases such as sphincter of Oddi dyskinesia and/or acute pancreatitis.

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