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# Cholesterol-fed Rabbits: Study of the Response of the Vas Deferens to Adrenergic and Non-adrenergic Stimulus and to a κ-Opioid Agonist

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**Abstract**

The aim of the present study was to analyse the contractility of the isolated vas deferens from hypercholesterolaemic rabbits; for this purpose we evaluated the contractile response induced by noradrenaline and by electrical stimulation.

A significant increase in the amplitude of adrenergic and non-adrenergic responses was observed in vas deferens from hypercholesterolaemic rabbits.

These data suggest an increase in the contractility of the smooth muscle in these animals.

Hypercholesterolaemia is a metabolic disturbance that induces changes in multiple physiological functions. Some of the most important modifications are associated with alterations of the contractility of the vascular smooth muscle and on the activity of the adrenergic system. Clinical and experimental studies have revealed that atherosclerotic vessels show augmented responses to certain vasoconstrictor agents, such as ergometrine, 5-hydroxytryptamine and histamine and increased sympathetic nervous system activity is associated with increased plasma cholesterol concentration (Smith et al 1992).

There is abundant information on the influence of hypercholesterolaemia, induced by administration of a diet enriched with cholesterol, on the contractility of the vascular smooth muscle. It has been demonstrated that the contractile responses to different kinds of stimulus, such as noradrenaline or KCl, are modified in isolated blood vessels. These modifications may be a decrease or an increase in response, depending on the vessels studied.

Nevertheless, there are sparse data about modifications of the contractile activity of non-vascular smooth muscle obtained from hypercholesterolaemic animals (Li et al 1994). And, as far as we know, there are no data testing the contractility of the vas deferens obtained from hypercholesterolaemic animals.

The aim of the present study was to analyse the contractility of the isolated vas deferens obtained from hypercholesterolaemic rabbits. For this purpose, we evaluated the contractile response induced by noradrenaline and by electrical stimulation.

In the rabbit vas deferens there are κ-opioid receptors, and the stimulation of these receptors is able to inhibit, in a dose-dependent manner, the contraction induced by continuous electrical stimulation (Oka et al 1981). Therefore, as a second goal, we measured the inhibition induced by U-50488H(*trans*-3,4-dichloro-*N*-methyl-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide hydrochloride), a selective κ-opioid agonist, to determine whether the hypercholesterolaemia modifies the effect of this drug, as cholesterol ester-treated membranes show modifications in opioid receptors.

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## Materials and Methods

The experiments were performed on male New Zealand White rabbits 3-months old at the time of delivery to the laboratory. The animals were initially fed on standard laboratory diet for at least 7 days after delivery to our laboratory. Diet and tap water were freely available.

Rabbits were then divided into two groups, one was fed on standard diet (control group) and the other (hypercholesterolaemic group), was fed on cholesterol-enriched diet (1% w/w) obtained from Usine d'Alimentation Rationelle (Paris, France). These diets were administered for 16 weeks. Weight was determined before starting the treatment, and then weekly. The body weight of the control animals (n = 10) averaged 2780 ± 63 g at the start of the experiment and 3997 ± 76 g after 16 weeks, and that of the cholesterol-fed animals (n = 9) averaged 2500 ± 204 and 3592 ± 174 g respectively.

At the end of the 16th week the animals were anaesthetized with ethyl ether and killed by exsanguination from common carotids. The vasa deferentia were carefully dissected as previously described (Oka et al 1981), and mounted in a 20-mL organ bath containing Krebs solution (mM) concentrations: NaCl 118; KCl 4·75; $CaCl_2$ 0·54; $KH_2PO_4$ 1·19; $MgSO_4$ 1·2; $NaHCO_3$ 25; glucose 11). This solution was continuously gassed with 95% $O_2$–5% $CO_2$. Tissues were kept under 0·5 g of resting tension at 32°C and then allowed to recover for 30 min.

Contractile responses, elicited either by electrical stimulation or by addition of noradrenaline, were isometrically recorded on a Grass model 7a polygraph.

Transmural stimulation was achieved using two different parameters.

*The effect of the κ-agonist*

Square pulses of 70 V, 0·1-ms duration and 0·3 Hz frequency were used. Cumulative concentration–response curves were constructed in a step-by-step manner after the response to the previous concentration had reached a plateau. The interval between application of increasing concentrations was 5 min. The inhibitory effect of U-50488H (100–800 nM) was evaluated as percent inhibition taking the last contraction before agonist addition as 100.

*Frequency–response (1–20 Hz) curve*

Pulses of 2-ms duration, supramaximal voltage, for 30 s each minute were used. The magnitude of the contractile response was evaluated as the mean of the force (g) recorded. This transmural stimulation of rabbit vas deferens causes a rapid twitch response (phasic response), followed by a sustained contractile response (tonic response). The data for each part of the response were separately evaluated. In both cases the pulses were delivered through two platinum ring electrodes.

Noradrenaline-induced contractions were obtained by the addition of increasing doses to the organ bath, starting with a concentration of 0·1 μM until the maximal amplitude of contraction was reached. The noradrenaline effect was estimated by considering the minimum concentration able to induce a contractile response (≥0·2 g) and the amplitude (g) of the maximal contractile response.

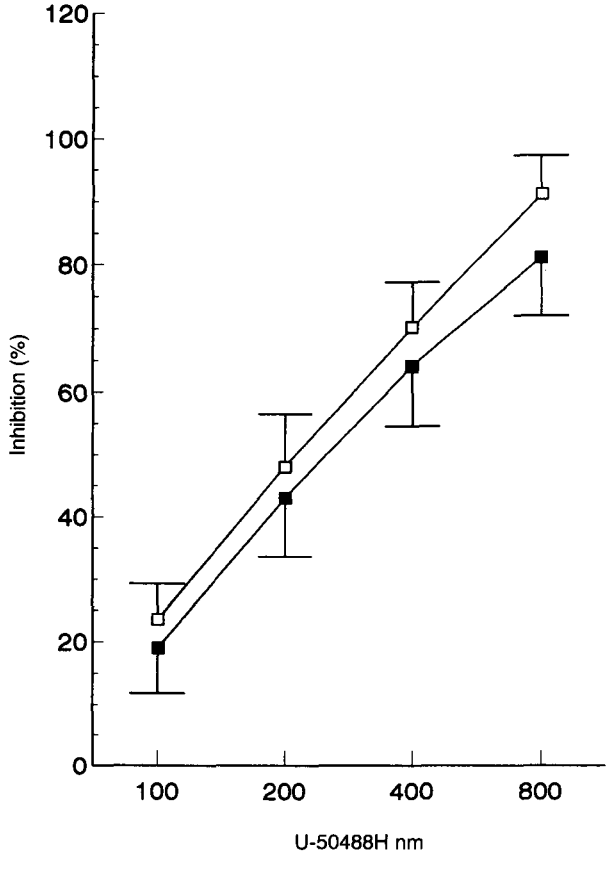


FIG. 1. Inhibition induced by U-50488H in electrically-induced contraction on vas deferens of control (□) and of cholesterol-fed (■) rabbits. Each point is the mean ± s.e.m. of six experiments.

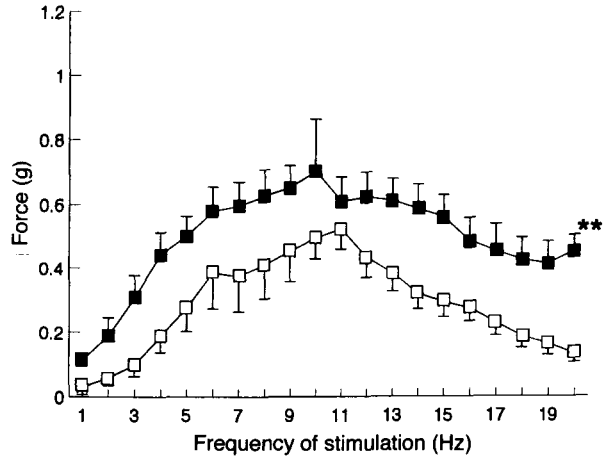


FIG. 2. Phasic contractions (g) obtained by increasing frequency of stimulation (Hz) in vas deferens of control (□) and of cholesterol-fed (■) rabbits. The dose-response curve to electrical stimulation was significantly shifted to the left in cholesterol-fed rabbits **($P < 0{\cdot}01$). Each point is the mean ± s.e.m. of six experiments.

Results are given as mean ± s.e.m. The data were analysed using two-way analysis of variance and Scheffe's test or Student's *t*-test. A level of probability $< 0{\cdot}05$ was accepted as statistically significant.

## Results

The inhibition of the twitch responses by U-50488H, was, as expected, dose-dependent. No differences were found between tissues obtained from control and hypercholesterolaemic rabbits, the ID50 values being 213·8 (n = 6) and 267·9 nM (n = 6), respectively (Fig. 1).

When frequency–response curves were constructed, a significant increase in the amplitude of the contraction in the vas deferens from hypercholesterolaemic rabbits was observed. This difference was found in both the phasic (Fig. 2) and tonic (Fig. 3) responses.

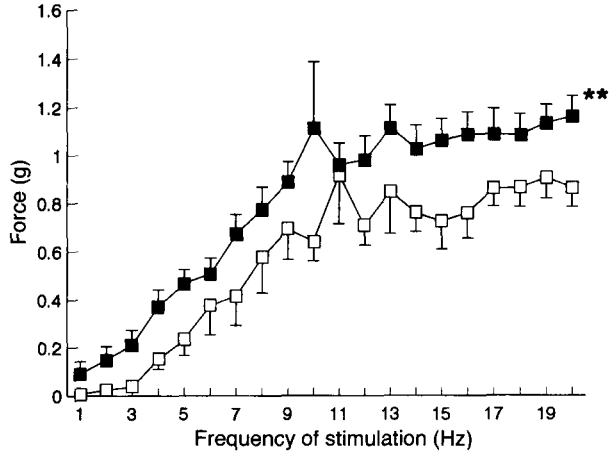


FIG. 3. Tonic contractions (g) obtained by increasing frequency of stimulation (Hz) in vas deferens of control (□) and of cholesterol-fed (■) rabbits. The dose-response curve to electrical stimulation was significantly shifted to the left in cholesterol-fed rabbits **($P < 0{\cdot}01$). Each point is the mean ± s.e.m. of six experiments.

A significant ($P < 0.05$) increase was also observed in the contractile response induced by noradrenaline. The mean amplitude of the responses was $1.5 \pm 0.26$ (n = 7) in control tissues and $2.5 \pm 0.19$ g (n = 6) in those of hypercholesterolaemic rabbits. The smallest concentration of noradrenaline able to induce contractile activity ($\geq 0.2$ g) was greater in control vas deferens than in tissues from hypercholesterolaemic rabbits, although the difference did not reach significance, the concentrations being $26.6 \pm 16$ (n = 7) and $3.8 \pm 1.1$ $\mu$M (n = 6), respectively.

## Discussion

One of the main characteristics of the rabbit vas deferens is the presence of $\kappa$-opioid receptors (Oka et al 1981). In this study we evaluated the effect of the $\kappa$-opioid agonist since it is well known that changes in the lipid configuration of the plasma membrane, and subsequent changes in the microviscosity, may induce changes in the responsiveness to opioids (Lazar & Medzihradsky 1992, 1993). Under our experimental conditions, we did not find significant modifications of the ability of U-50488H to inhibit the contractile response induced by electrical stimulation.

The vas deferens was used to study the non-vascular smooth-muscle effects of hypercholesterolaemia, since it represents a neurogenic preparation. This shows adrenergic and non-adrenergic contractions that can be eliminated by sympatholytic drugs, thus suggesting that both kinds of contractions are originated from adrenergic nerves (Allcorn et al 1986; Huston et al 1977). Consequently, this tissue has been widely employed to study the activity of drugs able to modify the vascular contractility, such as the agonists and antagonists of $\alpha$-receptors (Gillenwater et al 1978) or angiotensin (Trachte & Heller 1990; Trachte et al 1990). In spite of this, there was no information about the influence of hypercholesterolaemia on the contractile response induced by different kinds of stimulation.

As previously described, transmural stimulation of rabbit or guinea-pig vas deferens causes a phasic spike, followed by a sustained contractile response. The first part of the response is not reduced by $\alpha$-adrenoceptor antagonists (Swedin 1971) but is reduced by exogenous agonists by action at presynaptic $\alpha_2$-adrenoceptors (Vizi et al 1973). It is also eliminated by the ATP antagonist arylazido aminopropionyl ATP (Fedan et al 1981), by desensitization of the tissues to ATP-agonists (Sneddon & Burnstock 1984) and by the calcium-channel antagonist nifedipine (French & Scott 1983). These data suggest a purinergic origin for this first part of the contractile response.

The secondary tonic response is due to release of noradrenaline from adrenergic nerves (Johns et al 1976) and may be abolished by prazosin (Fedan et al 1981), phentolamine (Meldrum & Burnstock 1983) and reserpine (Kirkpatrick & Burnstock 1987).

Adrenergic blockade with guanethidine (Huston et al 1977) or with 6-hydroxy dopamine (Fedan et al 1981) inhibits both the twitch and sustained responses. It may be concluded that these effects are dependent on the adrenergic neurons.

Our results show that, in vas deferens obtained from hypercholesterolaemic rabbits, the force of the contraction was increased in the phasic response as well as in the secondary tonic response. Thus, no differences were found between the effect of hypercholesterolaemia on adrenergic and non-adrenergic phases of the contractile response.

Furthermore, the concentration of noradrenaline able to induce a contractile response is lower in hypercholesterolaemic rabbits and, moreover, the greatest amplitude of the contraction was found in this group of animals.

Taken together, these results indicate that there is an increase in the contractile response. This increase may be dependent on the contractility of the smooth muscle, since no differences were found between the adrenergic and non-adrenergic phases of the contraction induced by electrical stimulation. There is also an increase in the contractile response after the administration of exogenous noradrenaline.

Correlations could not be established between our data and those previously reported in vascular tissues. In blood vessels, hypercholesterolaemia may induce increases as well as decreases in the contractility induced by adrenergic or non-adrenergic mechanisms.

From our present data, we cannot suggest the final mechanism underlying this change in the muscle contractility, therefore this work must be considered as a first step in the analysis of the effects of hypercholesterolaemia in the contractility of non-vascular smooth muscle.

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