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FULL PAPER

Native and oxidised low-density lipoproteins modulate the vasoactive actions of soluble β -amyloid peptides in rat aorta

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Abbreviations: A β , β -amyloid; ACh, acetylcholine; AD, Alzheimer's disease; BBB, blood-brain barrier; EDTA, ethylenediaminetetraacetic acid; LDL, low density lipoprotein; NA, noradrenaline ; PBS, phosphate buffered saline; RAGE, receptor for advanced glycation end-products; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; Th-T, thioflavin T.

ABSTRACT

Cerebrovascular accumulation of β -amyloid ($A\beta$) occurs in aging and Alzheimer's disease (AD). Hypercholesterolaemia, which is associated with raised plasma low-density lipoprotein (LDL), may predispose to AD. Soluble $A\beta$ is found in the circulation and enhances vasoconstriction. Under conditions that may favour the formation of short $A\beta$ oligomers, as opposed to more severe polymerisation leading to $A\beta$ fibrillogenesis, we investigated the influence of low density lipoprotein (LDL) on the vasoactive actions of soluble $A\beta$ peptides. Thus, the actions of $A\beta$ 40 and $A\beta$ 42 in combination with native or oxidised LDL on vasoconstriction to noradrenaline (NA) and vasodilatation to acetylcholine (ACh) were examined in rat aortic rings. LDL, particularly when oxidised, potentiated NA-induced constriction when combined with soluble $A\beta$ 40 and, especially, $A\beta$ 42. Soluble $A\beta$ 40 reduced relaxation induced by ACh but $A\beta$ 42 was ineffective. Native and oxidised LDL also attenuated relaxation. Synergism occurred between oxidised LDL and $A\beta$ with respect to ACh-induced relaxation but not between native LDL and $A\beta$. We have shown for the first time that under conditions that may result in $A\beta$ oligomer formation LDL, particularly when oxidised, modulates the vascular actions of soluble $A\beta$ peptides to extents greater than those reported previously for fibrillar $A\beta$ preparations. Mechanisms whereby a treatable condition, namely hypercholesterolaemia, might contribute to the development of the cerebrovascular component of AD are indicated.

INTRODUCTION

β -amyloid ($A\beta$) is a 39-43 amino acid cytotoxic peptide that is derived from a 695-770 amino acid transmembrane amyloid precursor protein [1]. It is deposited as insoluble fibrils in both the brain parenchyma and in the walls of cerebral blood vessels in normal aging and, to an exaggerated extent, in Alzheimer's disease (AD). $A\beta$ occurs in a soluble form throughout the body, including in the circulation where it has been detected in plasma [2, 3] and platelets [4], primarily as the $A\beta$ 40 and $A\beta$ 42 isoforms [3]. Platelets have been shown to release significant quantities of $A\beta$ on aggregation, prompting the suggestion that these particles may represent a source of the $A\beta$ deposited in the tissues, including the cerebrovasculature [4-7].

It has been suggested that risk factors for coronary heart disease may also constitute risk factors for AD [8]. These factors include, hypercholesterolaemia, a common condition associated with elevated plasma levels of the atherogenic species low-density lipoprotein (LDL) and, significantly, of oxidised LDL [9]. We have reported previously that platelet $A\beta$ efflux is increased in hypercholesterolaemia [7]. Subsequently we demonstrated that native and, particularly, oxidised LDL promoted $A\beta$ polymerisation in a process involving β -pleated sheet formation [10,11]. Mullan and coworkers [12-14] have shown that soluble $A\beta$ peptides enhance constriction of rat aortic rings stimulated by phenylephrine and endothelin-1. We have obtained data indicating that fibrillar $A\beta$ 40 generated in the presence of native LDL and, to a greater extent, oxidised LDL enhance noradrenaline (NA)-induced contraction [11]. Recently, however, data have been presented indicating that $A\beta$ oligomers, rather than large $A\beta$ polymers (i.e. fibrils), may be particularly potent with respect to the toxicity of the $A\beta$ peptide and its cellular responses [15]. The aim of the current study, therefore, was to establish if $A\beta$ peptides, under conditions that may exist in vivo and could favour $A\beta$ oligomer formation, produce more marked effects on vessel tone than demonstrated previously for $A\beta$ 40 fibrils [11]. To this end the influence of soluble $A\beta$ 40 and $A\beta$ 42 on contraction induced by NA and relaxation stimulated by ACh in the presence and absence of native and oxidised LDL was examined. For comparison studies were also conducted with fibrillar (polymeric) $A\beta$ preparations. Thus, it was hoped that potential pathological biochemical mechanisms mediating the actions of $A\beta$ might be identified.

MATERIALS AND METHODS

Animals

Aortae from adult male Sprague Dawley rats (300-400g; Charles River, Wilmington, USA; total 72) were used in the experiments described. The animals were kept under conditions and the experiments were conducted in accordance with the *Animals (Scientific Procedures) Act 1986* published by the UK Home Office and the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Chemicals

Synthetic A β 40 and A β 42 peptides were purchased from Biosource International (Nivelles, Belgium) and stored at -85°C . All other chemicals were supplied by Sigma Chemical Co. (Poole, U.K.).

Plasma lipoprotein isolation

LDL was isolated from normal human plasma by sequential density gradient ultracentrifugation [16]. EDTA (240 μM) was included in all solutions used during the preparation of LDL as an antioxidant. After isolation lipoproteins were dialysed extensively against 0.9% w/v NaCl at 4°C .

Lipoprotein oxidation

Oxidation of LDL was carried out by dialysis against 1000 volumes of 0.9% NaCl containing 1 μM FeSO $_4$ at 4°C for 72h with a change of buffer every 24h. Oxidation was halted by dialysis against 1L of saline with 240 μM EDTA [10,11]. Native (non-oxidised) lipoproteins were subjected to a similar dialysis procedure in which the FeSO $_4$ was replaced by EDTA. Oxidised LDL exhibited enhanced migration on agarose electrophoresis (mobility relative to native LDL 1.5) indicating that oxidative modification of both the lipid and apoB components of LDL had occurred.

Lipoprotein characterisation

The protein content of lipoprotein fractions was determined as described by Wang and Smith [17]. Confirmation that successful oxidation of LDL had occurred was achieved by measuring lipoprotein peroxidation products. Total LDL peroxidation products were determined by measuring thiobarbituric acid reactive substances (TBARS) [18] and conjugated dienes, a specific lipid peroxidation product, measured

employing second derivative UV spectroscopy [19]. Comparing native LDL versus oxidised LDL, TBARS values were increased some 13-fold whilst conjugated dienes were increased some 20-fold.

A β 42 and A β 40 fibril formation.

A β 42 fibrillogenesis was assessed by thioflavin-T (Th-T) fluorescence spectroscopy [10,11,20]. Briefly, A β 42 peptide (final concentration, 1 mg/ml) dissolved in phosphate-buffered saline (PBS) was incubated at 37 °C for up to 1 h. At specific time intervals (0, 2.5, 5, 10, 15, 30, 45 and 60 min) aliquots (20 μ l) were removed, combined with Th-T reagent (2 μ M Th-T in 5mM glycine, pH 8.75) and the fluorescence measured (excitation and emission wavelengths 425 and 480 nm, band pass slits 5 and 10 nm, respectively). A β 40 fibrils were prepared as described previously [10,11].

Native and oxidised LDL and A β 42 and A β 40 fibrillogenesis

The actions of native and oxidised LDL on A β 42 polymerisation were determined by co-incubating the lipoproteins (2 mg/ml, final concentration) with soluble peptide (1 mg/ml, final concentration). Samples were incubated at 37 °C for up to 1 h and the Th-T fluorescence measured as outlined above.

A β 40 fibrils generated in the presence of LDL were obtained as outlined previously i.e. A β 40 was incubated with native or oxidised LDL for 120 hours at 37°C [10,11].

A β peptides and the contraction and relaxation of rat aortic rings

The influence of A β 40 and A β 42 peptides together with LDL on arterial contraction and relaxation was studied using the rat aortic ring preparation[11,21]. Ring segments 4 mm in length were prepared from aortae removed from 3-month old Sprague-Dawley rats. The rings were mounted in 10 ml organ baths containing Krebs bicarbonate buffer maintained at 37 °C and gassed with 95% O₂/ 5% CO₂. Changes in ring tension, as measured via a Grass FTO3 C pressure transducer, were recorded using a MacLab system (AD Instruments Pty. Ltd., Castle Hill, NSW, Australia). Aortic rings were equilibrated for 1h at a resting tension of 1.5 g. The rings were then treated with either saline or A β peptides (final peptide concentration in the organ bath, routinely 1 μ M, i.e. 4.3 μ g/ml and 4.5 μ g/ml for A β 40 and A β 42, respectively) for

15 min before contraction to NA, at a range of concentrations between 10^{-9} and 10^{-6} M, was assessed.

In experiments in which the effects of A β peptides on ACh-induced relaxation were examined, contraction to a full range of NA concentrations was first tested in order to establish the NA EC₅₀ dose. Then, following extensive washing (three changes of buffer), the aortic rings were challenged with the EC₅₀ dose of NA followed by increasing concentrations of ACh (10^{-9} to 10^{-5} M). When the influence of native and oxidised LDL on the contractile and relaxant responses to A β peptides were tested, lipoproteins were added so as to yield a final concentration in the organ-bath of 2 μ g protein/ml.

In the interests of clarity it should be noted that in experiments in which the actions of soluble A β peptides together with LDL were examined the components were added to the organ bath separately, whilst in experiments in which fibrillar A β preparations were investigated the components had been coincubated (120 hours) prior to their addition to the organ bath.

Data analysis

Data are presented as means \pm SEM and were evaluated statistically by Student's t-test and/or ANOVA with Bonferroni post-hoc analysis where appropriate.

Results

A β 42 fibrillogenesis

The incubation of solubilised A β 42 at 37 °C resulted in a time-dependent increase in Th-T fluorescence which peaked at 15-30 min (Table 1). Maximal values for fluorescence obtained at 30 min of incubation exceeded basal values 3.7-fold ($p < 0.001$). Native and oxidised LDL did not influence the rates or extents of A β 42 polymerisation significantly as determined by statistical analysis, although an apparent trend towards an increase was seen with native LDL.

Soluble A β 40, LDL and NA-induced vasoconstriction

Neither native nor oxidised LDL (2 μ g/ml protein, final concentration in the organ bath) altered NA-induced contraction of aortic rings significantly (Figure 1).

As reported previously [11] 1 μ M (4.3 μ g/ml) soluble (and fibrillar) A β 40 failed to influence vasoconstriction although raising the concentration to 5 μ M (21.6 μ g/ml) enhanced constriction ($p < 0.01$ and $p < 0.05$ with 10^{-9} and 5×10^{-9} M NA, respectively) (Figure 2A). In the present study, however, 1 μ M soluble A β 40 when

combined with native LDL and, particularly, oxidised LDL (2 μ g/ml) enhanced NA-induced constriction over the entire agonist concentration range (Figure 3). The increases in contraction were particularly marked and statistically significant for the lower agonist concentrations. Thus, with 10^{-9} , 5×10^{-9} and 10^{-8} M NA, soluble A β 40 plus native LDL increased contraction by 407% ($p < 0.001$), 104% ($p < 0.001$) and 51% ($p < 0.05$), respectively. Meanwhile, with soluble A β 40 plus oxidised LDL contraction was increased by 886% ($p < 0.001$), 185% ($p < 0.001$) and 122% ($p < 0.01$), respectively.

A β 42, LDL and vasoconstriction

Soluble and fibrillar A β 42 at a concentration of 1 μ M (4.5 μ g/ml) failed to enhance contraction stimulated by NA (Figure 2B). When, however, soluble A β 42 concentrations were raised to 5 μ M (22.6 μ g/ml) NA-induced responses were potentiated, although not to the extents observed for 5 μ M soluble A β 40 (Figures 2A and 2B). As seen with A β 40, but to a more marked degree, when soluble A β 42 was applied to aortic rings together with native LDL and, to a greater extent, oxidised LDL potentiation of the vasoconstrictive responses to NA occurred (Figure 4). Hence, with 10^{-9} , 5×10^{-9} , 10^{-8} , 5×10^{-8} , 10^{-7} , 5×10^{-7} and 10^{-6} NA, soluble A β 42 plus native LDL enhanced contraction by 1583 % ($p < 0.05$), 334 % ($p < 0.05$), 198 % ($p < 0.01$), 81 % ($p < 0.01$), 88 % ($p < 0.01$), 85 % ($p < 0.001$) and 83 % ($p < 0.001$). With soluble A β 42 plus oxidised LDL contraction was increased by 2250 % ($p < 0.001$), 514 % ($p < 0.001$), 327 % ($p < 0.01$), 148 % ($p < 0.01$), 147 % ($p < 0.01$), 134 % ($p < 0.01$) and 139 % ($p < 0.01$), respectively.

A β 42 fibrils generated in the presence of oxidised LDL yielded responses (across the complete noradrenaline concentration range) comparable to those obtained with soluble A β 42 plus oxidised LDL ($p < 0.001$) (Figure 5). With A β 42 fibrils prepared together with native LDL, however, the effects produced were similar to those observed with oxidised LDL/A β 42 fibrils (p values between < 0.01 and < 0.001), and greatly exceeded the responses seen with soluble A β 42 plus native LDL.

A β peptides, LDL and ACh-induced vasorelaxation

Soluble A β 40 at 1 μ M and 5 μ M inhibited relaxation induced by all ACh concentrations (p values ranged between < 0.05 and < 0.001) to similar extents, although at lower ACh concentrations 5 μ M A β 40 was more potent (Figure 6).

Fibrillar A β 40 and fibrillar A β 42 produced only marginal effects, whilst soluble A β 42 was without effect. Native and oxidised LDL produced marked inhibition of relaxation: with ACh concentrations between 5×10^{-9} and 10^{-5} M native LDL reduced relaxation by 33-46% (p values ranged between <0.01 and <0.001) and oxidised LDL by 20-34 % (p between <0.05 and <0.01) (Figure 7A). Synergy between soluble A β 40 and native LDL was not evident. With oxidised LDL plus A β 40 or A β 42 , however, data were obtained indicative of synergistic interactions between the two factors. Thus, with oxidised LDL plus soluble A β 40 and ACh concentrations ranging between 10^{-9} and 10^{-5} M relaxation was reduced by 43-80 %, whilst with oxidised LDL plus soluble A β 42 and 5×10^{-8} - 10^{-5} M ACh relaxation was reduced by 36-44 % (p values ranged between <0.05 and <0.01) (Figures 7B and 7C). A similar picture was revealed when the data from the experiments conducted with A β 42 fibrils generated in the presence of oxidised LDL were examined (data not shown). On the other hand, A β 40 fibrils generated in the presence of native or oxidised LDL only reduced relaxation to ACh marginally.

DISCUSSION

In the present study we have obtained data indicating that native and oxidised LDL interact with soluble A β 40 and A β 42 to enhance NA-induced vasoconstriction and reduce ACh-induced vasorelaxation. We suggest that such a mechanism could represent a means whereby hypercholesterolaemia might contribute to the development of AD, given that hypercholesterolaemia has been suggested as a risk factor for AD [8].

The accumulation of A β in the walls of cerebral blood vessels associated with cerebrovascular degeneration is a recognised phenomenon in AD [1,22]. Animal studies have shown that the administration of A β leads to cerebrovascular breakdown and impairment of the blood brain barrier (BBB), and A β traversing the BBB [23,24]. It has been suggested that the A β that accumulates in the cerebrovasculature in AD may originate in the circulation [4,6,7]. We are still, however, lacking important information concerning the mechanisms that mediate A β -induced vascular actions. Previously it was proposed that the formation of A β polymers, in the form of fibrils, may be a key factor in enhancing the toxicity of the peptide and that other factors present in the blood may promote this process [25]. Indeed, we reported that LDL enhances A β 40 fibrillogenesis and that this leads to increased vascular constriction and toxicity [10,11]. The conformational state of A β has been identified as being a

key element in influencing its vasoactivity, with peptide preparations containing moderate amounts of β -pleated sheet structure being the most effective [13]. More recently it was proposed that A β oligomers may be particularly potent with respect to the biological actions of the peptide [15]. We have speculated that A β 40 when incubated with LDL may yield peptide preparations enriched in the moderately β -pleated sheet conformation [11]. Data arising from the present study, particularly that relating to A β 40, can be interpreted as being consistent with the formation of oligomers. We would also suggest that under the conditions employed that A β oligomer formation may have been enhanced and that this may have been reflected by altered vascular responses. Thus, soluble A β 40 in combination with native or oxidised LDL produced vasoconstrictive effects that greatly exceeded those reported previously for A β 40 fibrils generated in the presence of LDL and which will possess a greater degree of β -pleated sheet structure [11]. In vivo, A β 40 is the form of the A β peptide that predominates in the circulation and is deposited primarily in the vasculature [4,5,26,27]. Given the relative slowness of A β 40 polymerisation compared to A β 42 it can be inferred that oligomer formation in the early stages of the polymerisation process would be favoured in preference to fibril formation with important consequences for vascular pathology and function. It is possible that A β 40 fibril formation proceeds via the generation of intermediate oligomers and that a “seeding process”, as proposed for A β fibrillogenesis, may be involved in the formation of toxic forms of A β [13]. The thioflavin-T assay routinely employed to assess A β polymer formation, however, lacks the acuity necessary to distinguish between different A β conformations and more sensitive analytical techniques will need to be utilised in order to establish unequivocally which forms of the peptide predominate under different experimental conditions, including those used in the current study.

The responses obtained with A β 42 were not as straightforward as those seen with A β 40. As reported for A β 40, soluble and fibrillar A β 42 when added to the organ bath, so as to achieve a final concentration of 1 μ M, failed to influence NA-induced aortic contraction [11,21]. Similar to soluble A β 40 [11,21] raising the concentration of soluble A β 42 to 5 μ M enhanced NA-stimulated constriction, albeit to a lesser extent than for A β 40: we have proposed that at higher A β concentrations, and under the influence of the law of mass action, the conditions in situ (i.e. in the organ bath) may be conducive to oligomer formation [21]. Clear differences between

A β 42 and A β 40 with respect to the vascular responses were, however, observed when the peptides were tested in combination with LDL. Thus, like A β 40, soluble A β 42 in combination with native LDL and, to a greater extent, oxidised LDL significantly enhanced constriction. However, unlike A β 40, A β 42 fibrils prepared with native and oxidised LDL yielded responses that in the case of the former exceeded those seen with the soluble peptide plus native LDL, and the latter were similar to those obtained with soluble peptide plus oxidised LDL. The apparent discrepancy between the data obtained with A β 40 and A β 42 may reflect differences as regards the kinetics of the polymerisation processes exhibited by the two peptides, the relative quantities of peptide oligomers generated or differences regarding the proportions of oligomers and polymers produced. Our results confirm that fibril formation in A β 42 preparations occurs at a much greater rate than for A β 40 [10, 11] and it is possible that this may result in a greater yield of oligomers compared to A β 40.

The concentrations of A β used in this study were greater than those reported for plasma [2], however, higher concentrations of A β peptide could occur locally, e.g. at the sites of platelet aggregation, and approach those that were employed. It cannot be ruled out that the results obtained with A β 40 or A β 42 in combination with LDL may reflect synergistic interactions between the two species with the activation of their relevant receptors. In the case of native LDL plus A β , activation of LDL receptors coupled with the activation of, for example, RAGE (receptor for advanced glycation end-products) by A β [28], may have occurred resulting in the threshold for activation of the individual receptors being exceeded and hence enhancement of NA-induced constriction. A similar mechanism may also have operated with oxidised LDL and A β , although, unlike native LDL, oxidised LDL is not a ligand for the LDL receptor but instead is taken up by the scavenger receptor (SR) [29].

The results of experiments in which the actions of A β peptides in combination with LDL on ACh-induced vasoconstriction were assessed reflected, to some extent, those obtained for vasoconstriction. Thus, as seen with vasoconstriction, the effects on ACh-induced relaxation produced by A β 40 or A β 42 together with LDL (i.e. attenuated vasorelaxation) were particularly marked when LDL was in an oxidised form.

In conclusion, we have shown for the first time that soluble A β peptides when combined with LDL under conditions that may lead to the generation of A β oligomers

produced effects on vessel tone that exceeded those reported previously by this laboratory for fibrillar A β preparations generated by incubating A β for extended periods in the presence of LDL [10]. Given reports that A β oligomers exhibit greater degrees of cytotoxicity than their polymeric counterparts [15] our findings are of particular relevance for the pathophysiological processes occurring in AD, especially in relation to the cerebrovasculature. Our data also add further weight to the theory that cardiovascular risk factors, and in the case of this study, hypercholesterolaemia, which is prevalent in the general population, may be involved in the aetiology of AD.

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Legends to Figures

Figure 1 Noradrenaline-induced constriction of rat aortic rings in the presence of 2µg/ml native LDL(■) or 2µg/ml oxidised LDL (▲). Control incubations were conducted in the absence of LDL (◇). Data are presented as mean ± SEM (n=5-7) and no significant differences were observed between treatment groups.

Figure 2 Noradrenaline-induced constriction of rat aortic rings in the presence of, A (left panel) soluble (1 µM, ■; 5µM, ●) or fibrillar Aβ 40 (1µM, ▲), or B (right panel) soluble (1µM, ■; 5µM, ●) or fibrillar Aβ 42 (1µM, ▲). Control incubations (◇) were conducted in the absence of Aβ peptides. Data are presented as mean ± SEM (n=3-7). With 5µM soluble Aβ 40 significant increases in contraction were seen with 10⁻⁹ M (**p<0.01) and 5 x 10⁻⁹ M (*p<0.05) noradrenaline.

Figure 3 Noradrenaline-induced constriction of rat aortic rings in the presence of soluble Aβ 40 (1µM) in combination with 2µg/ml native LDL (■) or 2µg/ml oxidised LDL (▲). Control incubations were conducted in the absence of either Aβ 40 or LDL (◇). Data are presented as mean ± SEM (n=7-9). Soluble Aβ 40 plus native or oxidised LDL significantly enhanced contraction induced by 10⁻⁹, 5 x 10⁻⁹ and 10⁻⁸ M noradrenaline (*p<0.05, **p<0.01, †p<0.001).

Figure 4 Noradrenaline-induced constriction of aortic rings in the absence (◇) or in the presence of soluble Aβ 42 (1µM) plus 2µg/ml native LDL (■) or oxidised LDL (▲). Data are presented as mean ± SEM (n=4-5). Soluble Aβ 42 plus native or oxidised LDL significantly enhanced contraction induced by 10⁻⁹, 5 x 10⁻⁹, 10⁻⁸, 5 x 10⁻⁸, 10⁻⁷, 5 x 10⁻⁷ and 10⁻⁶ M noradrenaline (*p<0.05, **p<0.01, †p<0.001).

Figure 5 Noradrenaline-induced constriction of aortic rings in the absence (◇, control) and presence of fibrillar Aβ 42 (1µM) generated in the presence of 2µg/ml native LDL (■) or oxidised LDL (▲). Data are presented as mean ± SEM (n=3-10). Contraction in the presence of Aβ 42/native LDL fibrils and Aβ 42/oxidised LDL

fibrils was significantly increased with all noradrenaline concentrations (** $p < 0.01$, † $p < 0.001$).

Figure 6 Acetylcholine-induced relaxation of aortic rings in the absence (◇, control) and presence of 1 μ M A β 40 (■) or 5 μ M A β 40 (▲). Data are presented as mean \pm SEM (n=4-7). Differences between control and A β 40 treated aortic rings occurred with all acetylcholine concentrations (* $p < 0.05$, ** $p < 0.01$, † $p < 0.001$).

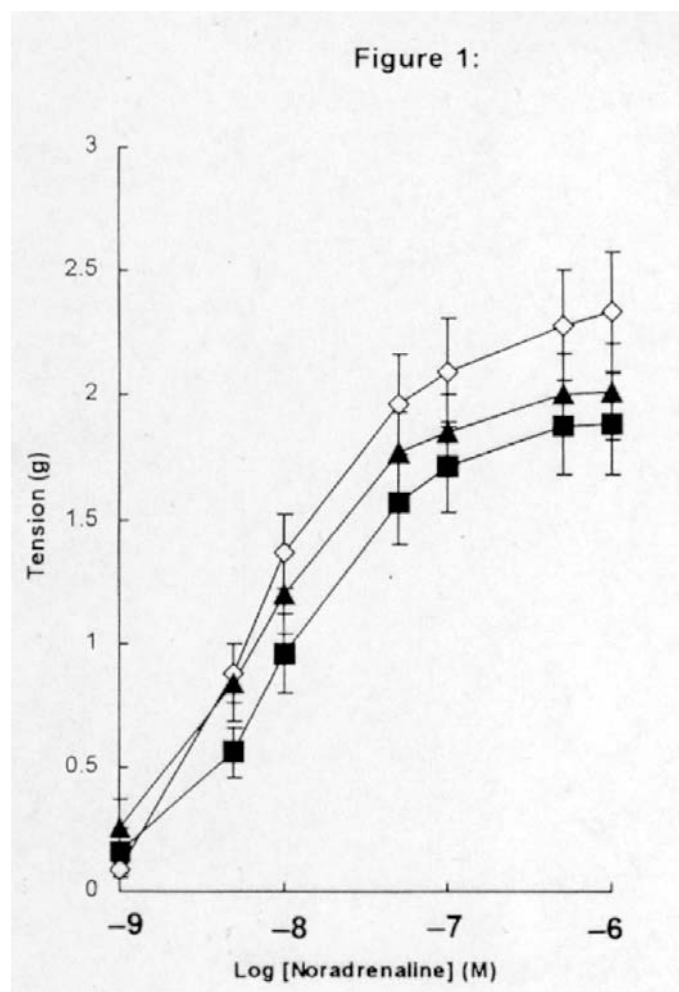
Figure 7 Acetylcholine-induced relaxation of rat aortic rings in the presence and absence of 1 μ M A β 40 or A β 42 in combination with 2 μ g/ml native or oxidised LDL. A (left panel): ◇ control (LDL absent); ■ native LDL; ▲ oxidised LDL. B (middle panel): ◇ control (A β 40 plus LDL absent); ■ A β 40 plus native LDL; ▲ A β 40 plus oxidised LDL. C (right panel): ◇ control (A β 42 plus LDL absent); ■ A β 42 plus native LDL; ▲ A β 42 plus oxidised LDL. Data are presented as mean \pm SEM (n=3-8). Native and oxidised LDL with ACh concentrations between 5×10^{-9} M and 10^{-5} M, oxidised LDL plus A β 40 with ACh concentrations between 10^{-9} M and 10^{-5} M, and oxidised LDL plus soluble A β 42 with ACh concentrations between 5×10^{-8} M and 10^{-5} M yielded values which differed from those obtained under control conditions (* $p < 0.05$, ** $p < 0.01$, † $p < 0.001$).

Table 1

A β 42 fibrillogenesis in the presence and absence of native or oxidised LDL

Time (min)	Fluorescence (AU)		
	A β 42	A β 42 + Native LDL	A β 42 + Oxidised LDL
0	40 \pm 3.8	36.9 \pm 3.0	35.4 \pm 2.3
2.5	78.5 \pm 11.5	101.5 \pm 14.6	77.7 \pm 6.2
5	117.7 \pm 8.5	122.3 \pm 15.4	110.8 \pm 6.9
10	129.2 \pm 4.6	143.1 \pm 9.2	130.8 \pm 6.9
15	139.2 \pm 6.2	150.8 \pm 6.1	144.6 \pm 6.2
30	146.0 \pm 4.6	154.6 \pm 5.4	146.0 \pm 4.6
45	146.0 \pm 7.7	153.1 \pm 3.1	143.8 \pm 5.8
60	146.0 \pm 6.9	154.6 \pm 1.5	148.5 \pm 5.4

Data are presented as mean fluorescence \pm SEM (n = 6).



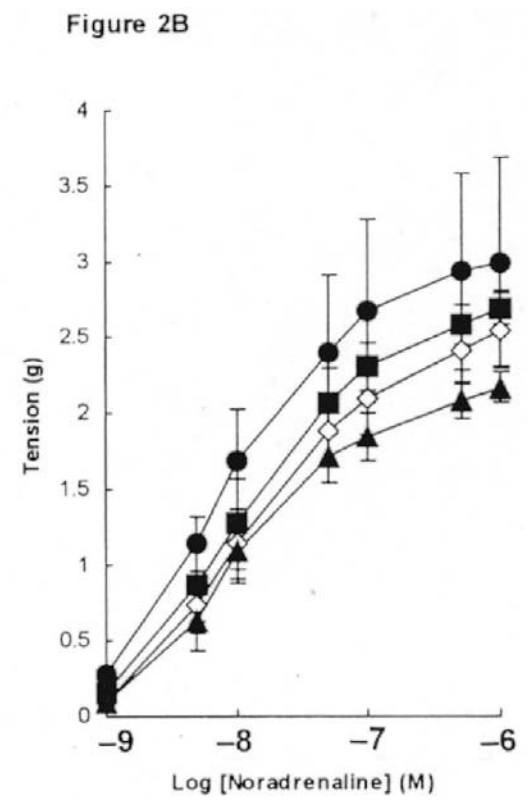
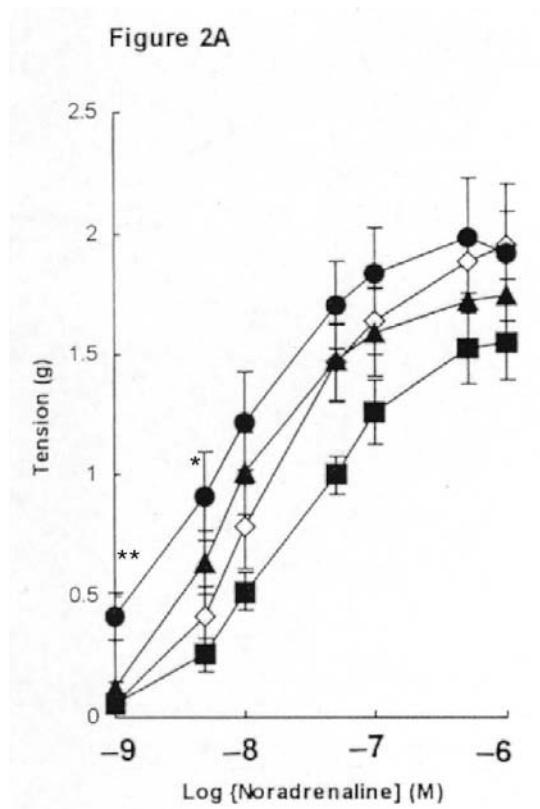


Figure 3

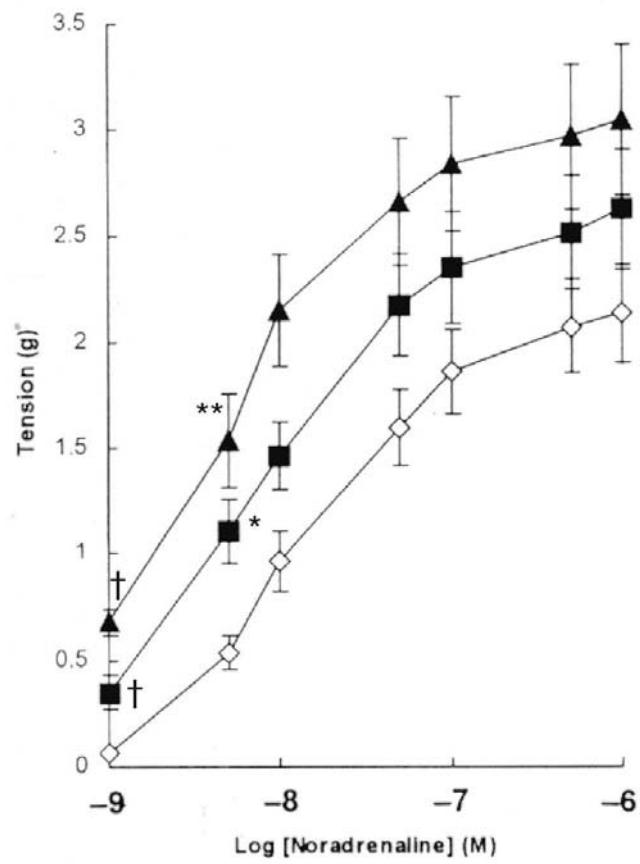


Figure 4

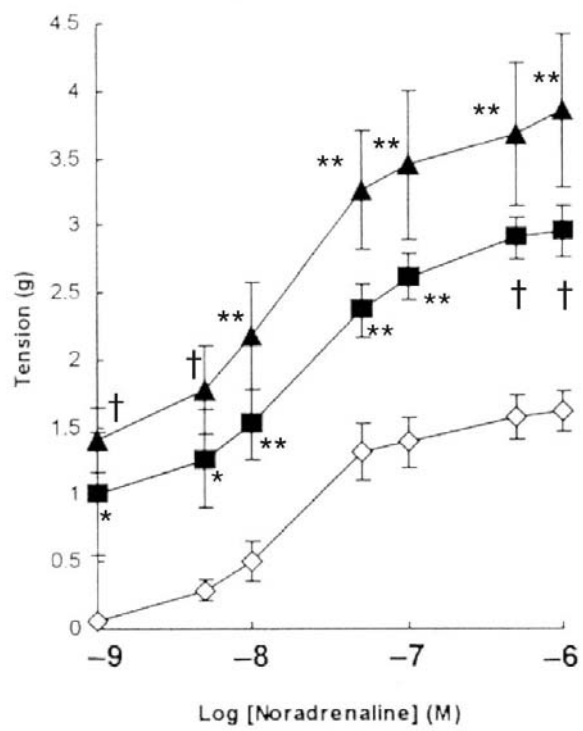


Figure 5

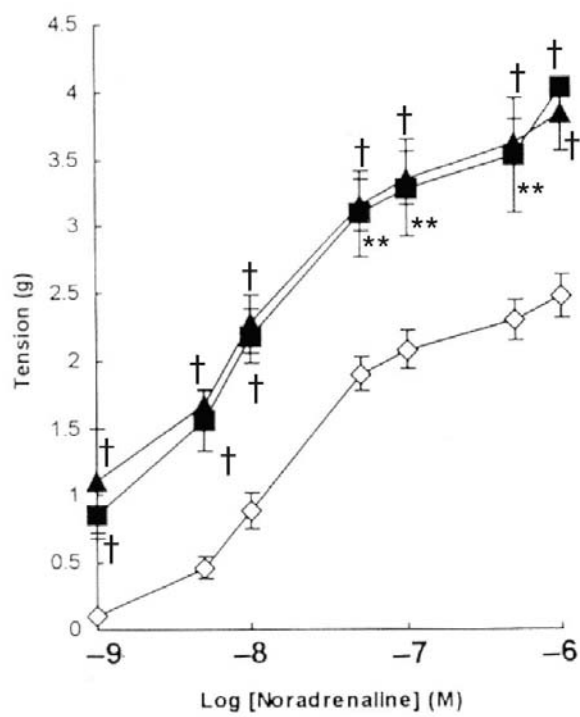


Figure 6

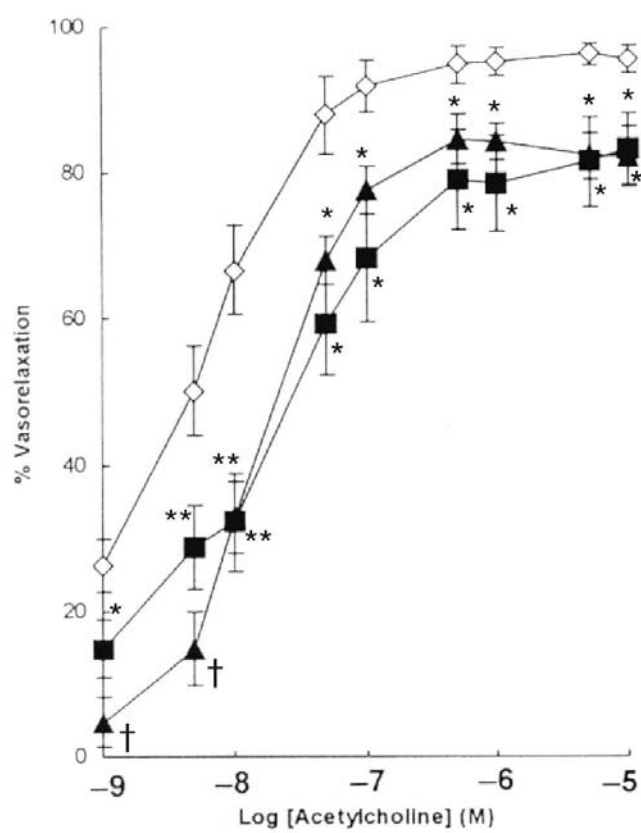


Figure 7

