Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2369826 C 2011/01/25

(11)(21) 2 369 826

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 2000/03/20
(87) Date publication PCT/PCT Publication Date: 2000/10/19
(45) Date de délivrance/Issue Date: 2011/01/25
(85) Entrée phase nationale/National Entry: 2001/10/09
(86) N° demande PCT/PCT Application No.: EP 2000/002453
(87) N° publication PCT/PCT Publication No.: 2000/061742
(30) Priorité/Priority: 1999/04/07 (DE199 15 485.6)

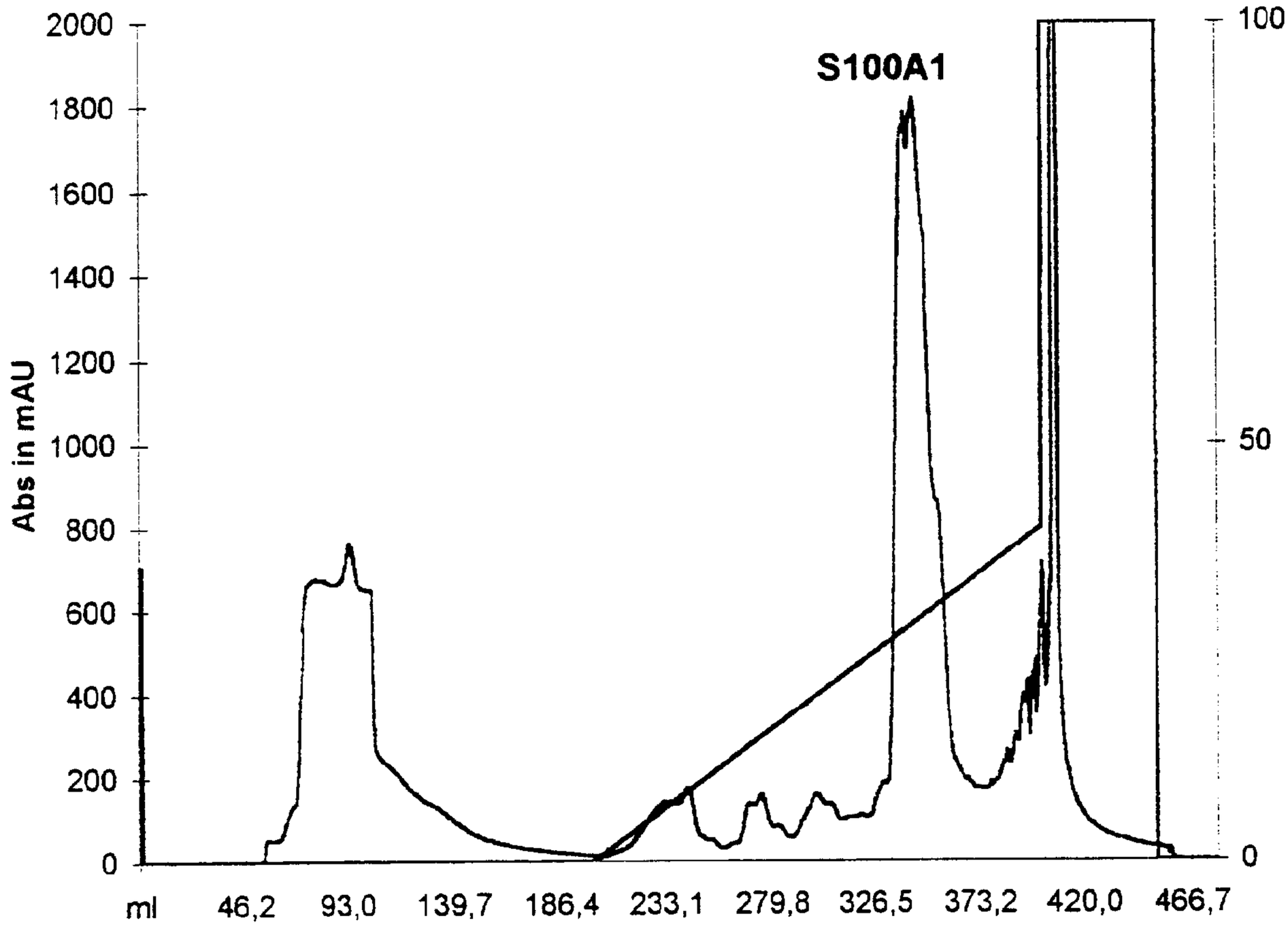
(51) Cl.Int./Int.Cl. *C12N 15/86* (2006.01), *A61K 31/7088* (2006.01), *A61K 38/17* (2006.01), *A61K 48/00* (2006.01), *A61P 9/10* (2006.01), *C07K 14/47* (2006.01), *C12N 15/12* (2006.01), *C12N 15/85* (2006.01), *A61K 38/00* (2006.01)

(72) Inventeurs/Inventors:
KATUS, HUGO A., DE;
REMPPIS, ANDREW, DE;
MOST, PATRICK, GE

(73) Propriétaires/Owners:
REMPPIS, ANDREW, DE;
MOST, PATRICK, GE

(74) Agent: BERESKIN & PARR LLP/S.E.N.C.R.L.,S.R.L.

(54) Titre : TRAITEMENT DE L'INSUFFISANCE CARDIAQUE
(54) Title: THERAPY OF CARDIAC INSUFFICIENCY



(57) Abrégé/Abstract:
The invention relates to medicaments for treating cardiac power failure. Said medicaments contain a therapeutically effective quantity of one or more S100 protein(s) or one or more mutant or fragments of the same, or contain one or more nucleic acid sequence(s) which code(s) for these amino acid sequences and which are optionally integrated in one or more gene transfer vectors.

CA 02369826 2001-10-09

(12) NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT) VERÖFFENTLICHTE INTERNATIONALE ANMELDUNG

(19) Weltorganisation für geistiges Eigentum
Internationales Büro

(43) Internationales Veröffentlichungsdatum
19. Oktober 2000 (19.10.2000)

PCT

(10) Internationale Veröffentlichungsnummer
WO 00/61742 A3

(51) Internationale Patentklassifikation[7]: C12N 15/12, A61K 38/17, 48/00, C07K 14/47, A61P 9/10

(21) Internationales Aktenzeichen: PCT/EP00/02453

(22) Internationales Anmeldedatum:
20. März 2000 (20.03.2000)

(25) Einreichungssprache: Deutsch

(26) Veröffentlichungssprache: Deutsch

(30) Angaben zur Priorität:
199 15 485.6 7. April 1999 (07.04.1999) DE

(71) Anmelder und
(72) Erfinder: **KATUS, Hugo, A.** [DE/DE]; Domhof 23, D-23909 Ratzeburg (DE). **REMPPIS, Andrew** [DE/DE]; Moltkestrasse 8, D-23564 Lübeck (DE).

(74) Anwälte: **WEBER-QUITZAU, Martin** usw.; Uexküll & Stolberg, Beselerstrasse 4, D-22607 Hamburg (DE).

(81) Bestimmungsstaaten *(national)*: CA, JP, US.

(84) Bestimmungsstaaten *(regional)*: europäisches Patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

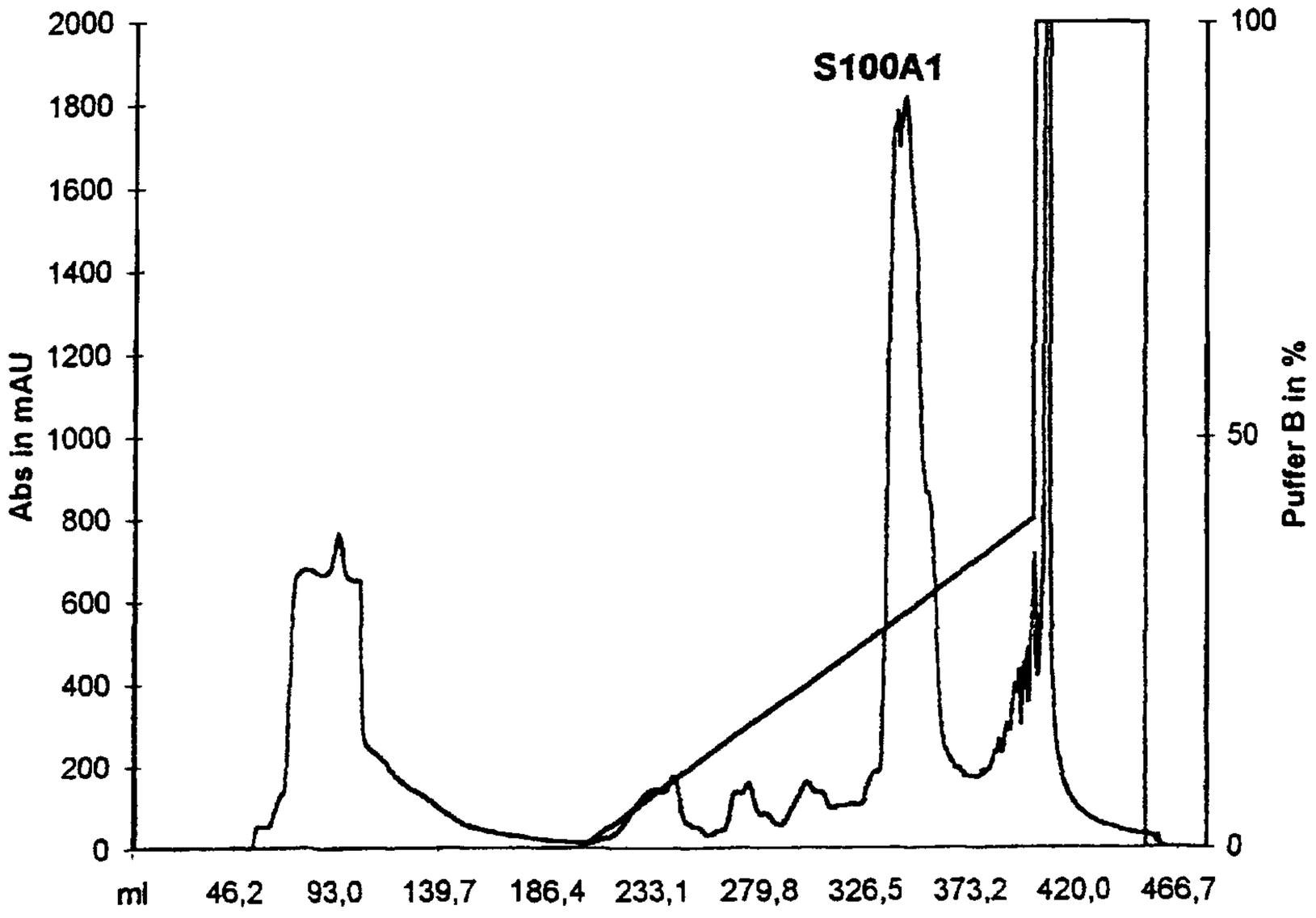
Veröffentlicht:
— *Mit internationalem Recherchenbericht.*

(88) Veröffentlichungsdatum des internationalen Recherchenberichts: 29. März 2001

[Fortsetzung auf der nächsten Seite]

(54) Title: TREATMENT OF CARDIAC POWER FAILURE USING S100 PROTEINS

(54) Bezeichnung: THERAPIE DER HERZINSUFFIZIENZ DURCH VERWENDUNG DER S100-PROTEINEN



(57) Abstract: The invention relates to medicaments for treating cardiac power failure. Said medicaments contain a therapeutically effective quantity of one or more S100 protein(s) or one or more mutant or fragments of the same, or contain one or more nucleic acid sequence(s) which code(s) for these amino acid sequences and which are optionally integrated in one or more gene transfer vectors.

[Fortsetzung auf der nächsten Seite]

WO 00/61742 A3

WO 00/61742 A3

Zur Erklärung der Zweibuchstaben-Codes, und der anderen Abkürzungen wird auf die Erklärungen ("Guidance Notes on Codes and Abbreviations") am Anfang jeder regulären Ausgabe der PCT-Gazette verwiesen.

(57) Zusammenfassung: Die vorliegende Erfindung betrifft Arzneimittel zur Behandlung von Herzinsuffizienz, die eine therapeutisch wirksame Menge eines oder mehrerer S100-Protein oder einer oder mehrerer Mutanten oder Fragmente derselben oder für diese Aminosäuresequenzen kodierende Nukleinsäuresequenz(en), gegebenenfalls in einen oder mehrere Gentransfervektoren integriert, enthalten.

Therapy of cardiac insufficiency

The present invention relates to medicaments for treating cardiac insufficiency which contain a therapeutically effective quantity of one or more S100 proteins, one or more mutants or fragments of same or nucleic acid sequence(s) coding for these amino acid sequences, optionally integrated into one or more gene transfer vectors.

Changes in intracellular Ca^{2+} homeostasis play a pathophysiologically pivotal role in cardiac insufficiency at the molecular level. Gwathmey et al (1) were the first to detect prolonged intracellular Ca^{2+} transients in contracting heart-muscle preparations of patients with end stage heart failure. Against the backdrop of an increased diastolic Ca^{2+} level and reduced systolic Ca^{2+} *peaks* (2), this finding was interpreted as an indication of a dysfunction of the sarcoplasmic reticulum (hereafter abbreviated to SR) which correlates at the haemodynamic level with an inverse force-frequency relationship of the myopathic heart muscle (3).

Of central importance here is the reduced re-uptake of Ca^{2+} into the SR during the diastole through the Ca^{2+}ATPase (Ca^{2+} pump of the SR which pumps the Ca^{2+} out of the

cytosol into the SR against a concentration gradient of 1:10,000). This leads on the one hand to a disturbed relaxation of the heart muscle during the diastole and to an associated reduction in Ca^{2+} release during the systole and thus to a reduced force development of the heart muscle. Among others, this observation is based on the fact that insufficient hearts have reduced cAMP levels (4). The cAMP-dependent phosphorylation of phospholamban is a precondition for activation of the Ca^{2+}ATPase of the SR.

One of the existing strategies for improving contractility therefore aimed at an increase in intracellular cAMP levels through the administration of phosphodiesterase inhibitors. Although this pharmacological approach leads to an improvement in cardiac performance in the short term, this therapy option was left for the chronic treatment of cardiac insufficiency, as it leads to a 53% excess mortality of the patients examined compared with placebo.

A further strategy pursued to date consists of using more effectively the reduced Ca^{2+} supply during the systole through a sensitization of the contractile apparatus with Ca^{2+} sensitizers that allow an increased strength development of the contractile apparatus for the same Ca^{2+}

concentration. Clinical studies carried out to date with pimobendan were disappointing however, as no significant improvement of the heart function compared with placebo was to be documented (7). There are still no clinical data for the inhomogenous group of new Ca^{2+} *sensitizers*. However, with some Ca^{2+} sensitizers, this therapeutic approach leads, through an increased Ca^{2+} sensitivity of the contractile apparatus, to a deteriorated relaxation so that the therapeutic advantage of an increased systolic strength development is called into question by a disturbance of the diastole (8).

In the search for further molecular causes for a restricted function of the SR within the framework of cardiac insufficiency, a reduced Ca^{2+}ATPase activity in crude membrane preparations (crude membranes) of insufficient hearts was able to be measured for different animal models and also for humans, so that a changed protein composition of the SR was assumed to be the cause. However, studies of the gene expression of the SR proteins phospholamban, Ca^{2+}ATPase and ryanodine receptor yielded varying and partly contradictory data. Thus, various work groups (9, 10, 11) found a significant reduced expression of the genes coding for phospholamban and Ca^{2+}ATPase also at protein level, whilst Movsesian et al (12) and Schwinger et al (13) documented no

significant expression differences and Arai et al (14) found a differential expression in the course of hypertrophy development. Although a significantly lower Ca^{2+}ATPase activity was detected in crude membrane preparations of terminally insufficient hearts (15), this finding was incomprehensible for highly purified membrane preparations of the sarcoplasmic reticulum (12). To date, the contradictory results have been attributed to varying analysis methods (12).

The object of the present invention is therefore to provide medicaments for the treatment of cardiac insufficiency, in particular for the treatment or improvement of the pumping capacity of the heart, restricted within the framework of cardiac insufficiency. The medicaments are preferably furthermore intended to increase heart power in general and to be suitable for a treatment of acute and chronic cardiac insufficiency.

This object is achieved in general according to the invention by using S100 proteins, the proteins either being used directly as active ingredient or an overexpression of S100 proteins in the heart muscle being effected through a gene-therapy approach. In particular, the subjects of claims 1 to 37 serve to achieve the object.

S100 proteins belong like calmodulin to the group of Ca^{2+}-binding proteins with EF-hand Ca^{2+}-binding motifs of which over 200 are now known. The family of the S100 proteins itself comprises 19 members of which 13 are coded on a narrow gene cluster on chromosome 1q21. Functionally, these proteins are incorporated into the regulation of cell differentiation, cell cycle regulation, signal transduction as well as Ca^{2+} homeostasis (16). In contrast to the ubiquitously expressed calmodulin, S100 proteins have a tissue-specific expression pattern (17). They therefore translate the Ca^{2+} signal into a tissue-specific response by interacting with specific target proteins after binding of Ca^{2+} to their EF hands (16). S100 proteins have a strongly preserved amino acid sequence with high homology within the S100 family. The amino acid and cDNA sequences are shown in the sequence protocol as SEQ ID NO: 1 to 30 (numerical code <210>).

By S100 proteins within the meaning of the invention are meant the complete native proteins, mutants of the S100 proteins, peptides (fragments) of the S100 proteins or peptide mutants (with a homology of at least 60%, preferably at least 90% and particularly preferably at least 95%) as well as recombinantly prepared proteins or peptides or mutants or synthetic peptides or mutants.

According to a particular version of the invention, the S100 protein is S100β (S100B), S100A1, S100A2, S100A4 or S100A6 (cf. Schäfer et al., Genomics 23 (1995) 638-643). In the following, by the term "S100 protein" or "protein" is alternatively also meant the named mutants or peptides. Because of the species-general homology of the S100 proteins according to the invention, proteins and sequences coding for them of any species (such as e.g. pig, cattle etc.) can be used, the corresponding human sequences being most preferred.

The subject of the present invention is therefore medicaments (pharmaceutical preparations) for the treatment of heart diseases with reduced contractility force of whatever cause (primary and secondary cardiomyopathies), which contain a therapeutically effective quantity of one or more S100 proteins (preferably S100β, S100A1, S100A2, S100A4 or S100A6), one or more mutants or fragments of same, which is optionally formulated with pharmaceutically compatible auxiliaries and/or supports.

According to a preferred version of the invention, the S100 protein S100A1, which contains the amino acid sequence shown in SEQ ID NO: 2, and the preferred fragments contain the amino acid sequences shown in SEQ

ID NO: 32, 34 and 36. Predominantly hydrophobic peptides can be extended to improve the solubility at the C- or N-terminal with hydrophilic amino acids. Preferably, according to the invention, the truncated and/or modified fragments according to SEQ ID NO: 37, 38 and/or 39 can be used.

Either single proteins, mutants or peptides of those named serve as active ingredients, but any mixtures of same can also be used, such as e.g. a combination of at least two of the peptides shown in SEQ ID NO: 32, 34, 36, 37, 38 and 39.

Within the framework of the present invention, by primary cardiomyopathies are meant hereditary cardiomyopathies and cardiomyopathies due to spontaneous mutations, by secondary cardiomyopathies are meant ischaemic cardiomyopathy due to an arteriosclerosis, dilatative cardiomyopathy due to an infectious or toxic disease of the myocardium, hypertensive heart disease due to a pulmonary-arterial and/or an arterial hypertonia, structural heart diseases due to rhythm disturbances and diseases of the heart valves, the primary and secondary myopathies being the cause of the cardiac insufficiency. The present application therefore furthermore relates to medicaments for the treatment of cardiac insufficiency.

For application, a direct injection of purified S100 protein (or mutants or peptide) either intravenously, intraarterially or intracoronally and/or long-term also the oral administration of recombinant protein or synthetic peptide analogues is possible.

Within the framework of the present invention, it was surprisingly found that in the cell culture model, after treatment with S100 protein, in particular S100A1 protein, and after S100 gene addition (in particular S100A1), an increase in the shortening and relenghtening speed of cultivated myocardial cells is to be documented, which correlates with changed intracellular Ca^{2+} transients in the sense of an increased systolic Ca^{2+} release from the SR and an accelerated Ca^{2+} re-uptake into the SR (see examples). These results were also able to be confirmed with other proteins of the S100 family.

The invention therefore furthermore relates to medicaments for the treatment of primary and secondary cardiomyopathies as well as cardiac insufficiency, which contain nucleic acid sequence(s) coding for one or more S100 proteins or one or more mutants or fragments of same, the nucleic acid(s) optionally being formulated with pharmaceutically compatible auxiliaries and/or

supports. These nucleic acid sequence(s) can also be contained in one or more gene transfer vectors. The nucleic acid sequences coding for the S100 proteins used according to the invention are reproduced in SEQ ID NO: 1 to 35, SEQ ID NO: 1 (S100A1 cDNA) being particularly preferred. According to a preferred version of the invention, a combination of at least two of the nucleic acid sequences coding for SEQ ID NO: 32, 24, 36, 37, 38 and 39 (in particular SEQ ID NO: 31, 33, 35 as well as sequences coding for SEQ ID NO: 37, 38 and 39) are used. In the gene-therapy approach, one or more gene transfer vectors comprising this combination are considered, i.e. the nucleic acid sequences named can either be cloned into several vectors (e.g. each singly), or the combination of the coding sequences can be contained in a single vector.

The nucleic acids or gene transfer vectors are optionally formulated for intravenous, intraarterial, intracoronal or oral application. A use of DNA in liposomal fractions represents a further possibility for application, even if it displays a lower transfection efficiency.

The subject of the present invention is thus furthermore the use of a construct which makes possible an overexpression of S100, preferably of S100A1, in the

heart muscle. Preferred is a viral construct which contains the DNA of an S100 protein, in particular of the S100β, S100A1, S100A2, S100A4 and the S100A6 protein, and which is preferably applied coronarally via a coronary catheter, displaying the highest transfection efficiency after this application.

The present invention thus furthermore relates to a process for the preparation of a gene transfer vector coding for one or more S100 proteins or one or more mutants or fragments of same, in which nucleic acid sequence(s) coding for the protein(s), the mutant(s) or the fragment(s) is (are) cloned into one or more vectors suitable for gene therapy.

The coding nucleic acid sequence is preferably selected from the group of the nucleic acid sequences represented in SEQ ID NO: 1 to 30. According to a particularly preferred version of the invention, the S100 DNA (sequence coding for S100A1 according to SEQ ID NO: 1), or the nucleic acid sequences coding for the fragments according to SEQ ID NO 32, 34, 36, 37, 38 and/or 39 (in particular SEQ ID NO: 31, 33, 35 as well as sequences coding for SEQ ID NO: 37, 38 and 39) are used for the preparation of the gene transfer vectors or for direct application.

In principle, several viral vector systems are suitable for carrying out a transfection of the heart tissue with S100 DNA. Recent vector systems (G. Bilbao et al., FASEB J. 11 (1977) 624-634; A. Amalficano et al. J. Virol. 72 (1998) 926-933) can produce an improvement in gene-therapy efficiency due to the immunological side effects still being only minimal compared with known adenoviral vectors. Gene-therapy efficiency can be further optimized by using strong heart-specific promoters, such as e.g. through a α-myosin heavy-chain promoter.

The effect of the S100 proteins can furthermore be increased by using corresponding sense oligonucleotides but the use of anti-sense oligonucleotides for S100 inhibitory substances can also be considered. Sense oligonucleotides (or anti-sense oligonucleotides of antagonistically acting proteins/peptides) or S100 proteins can be transferred directly into the vascular wall of the coronary arteries. Using a balloon system with a gel surface as carrier substance for oligonucleotides or proteins, the transfer of these molecules into the endothelial layer of the vessel accompanied by inflation of the balloon is achieved. This application method is however restricted to the influencing of cardial contractility by improving the

vascular function in the sense of an improvement of the endothelial or smooth-muscular function, as only a local increase in the S100 protein concentration can be achieved herewith.

The transfection with S100A1 DNA leads to an increased concentration of S100A1 protein in the heart tissue, an underexpression of S100A1 which occurs within the framework of the cardiac insufficiency thus being treated causally by gene therapy and the function of this protein guaranteed again. The positively inotropic and lusitropic effect of S100A1 in the heart tissue is to be emphasized. Under S100A1 gene therapy of cultivated myocardial cells, of reconstituted heart tissue (18), and in an in-vivo model of the rabbit heart, after overexpression of S100A1, there is a significantly increased velocity of contraction of at least 20% (positive inotropy) and an accelerated relaxation (positive lusitropy). This effect is based on an improved function of the sarcoplasmic reticulum, which is characterized by an accelerated as well as increased Ca^{2+} release from the SR as well as re-uptake of Ca^{2+} back into the SR. This is documented in some hitherto unpublished tests with bioluminescence-supported analysis of the intracellular Ca2+ transients (see examples).

The advantage of this invention is in particular that the dysfunction of the SR which is pathognomonic for cardiac insufficiency is causally treated.

Within the framework of the present invention therefore, not only are medicaments for the treatment of (chronic) cardiac insufficiency made available, but also medicaments for the treatment of heart diseases which are understood to be the cause of a cardiac insufficiency, such as primary cardiomyopathies (e.g. hereditary cardiomyopathies, cardiomyopathies due to spontaneous mutations) and secondary cardiomyopathies (e.g. ischaemic cardiomyopathy due to an arteriosclerosis, dilatative cardiomyopathy due to an infectious or toxic disease of the myocardium, hypertensive heart disease due to a pulmonary-arterial and/or arterial hypertonia, structural heart diseases due to rhythm disturbances, disease of the heart valves, etc.).

A further advantage of this invention is that further functions are described for S100, in particular for S100A1 which can significantly broaden the gene-therapy approach to the treatment of cardiac insufficiency.

Studies by Donato and by Garbuglia et al (19, 20) showed an inhibition of the polymerization of microtubuli and of

intermediate filaments by S100A1 in biochemical reconstitutive assays. Immunohistochemical analyses by Schaper et al (21) document a significant increase in the microtubule network and of random intermediate filaments in explanted hearts of patients with cardiac insufficiency of NYHA class IV (New York Heart Association IV) which leads to an increased viscous load of the insufficient myocardium and thus to a reduced contraction speed (22). The therapeutic overexpression of S100A1 can thus prevent a hyper cytoskeletal crosslinking and improve the contraction conditions by reducing the viscous load.

Data from Baudier et al (23) finally document an inhibition of protein kinase C through S100A1. As the activation of protein kinase C has a key position in the signal transduction cascade of the hypertrophy process leading to insufficiency (24), an inhibition of protein kinase C through increased S100A1 tissue levels in the insufficient myocardium could be of great therapeutic significance for a normalization of the signal transduction of the insufficient heart.

The following examples, figures and the sequence protocol are intended to explain the invention in more detail without limiting it to same.

Example 1

Purification of recombinant S100A1

Human recombinant S100A1 was prepared in genetically modified E. coli. For this purpose, the cDNA of S100A1 was cloned into a pGEMEX expression vector, competent E. coli were transformed with this vector and selected via an ampicillin resistance contained in the vector. The genetically modified bacteria were stored until their use in 50% glycerin at -80°C and then mixed according to the following method: 3 ml LB medium with 100 µg/ml ampicillin were seeded from the storage vessel with an eyelet and incubated overnight at 37°C. The following day, the overnight culture was added to 250 ml LB medium with 100 µg/ml ampicillin and 30 µg/ml chloramphenicol. The optical density was measured every 30 minutes, and with OD > 0.5 120 µl 1 M IPTG were added. After a further 3-4 hours, the medium was centrifuged off and the pellets were frozen and thawed 3-4 times to lyse the bacteria.

The bacterial pellets were homogenized in extraction buffer (25 mM tris-HCl, pH 7.5, 50 mM KCl, 1 mM PMSF, 5 mM EDTA) by means of ultrasound. The supernatants were brought to an end concentration of 2 M with saturated

ammonium sulphate solution, and centrifuged off. The supernatant of ammonium sulphate precipitation was applied to a HiTrap octyl-sepharose column (Phamacia, Freiburg), which was equilibrated in advance with buffer A (25 mM tris-HCl, pH 7.5, 2 mM CaCl2). The S100A1 bound to the column was eluted with a stepped gradient to buffer B (25 mM tris-HCl, pH 9.5, 5 mM EGTA). To achieve a purity of more than 95%, the S100 eluate was then subjected to an anion exchange chromatography. The sample was deposited on a HiTrapQ column (Pharmacia) previously equilibrated in buffer A (25 mM tris-HCl, pH 7.5) and eluted through a linear gradient to 40% buffer B (25 mM tris-HCl, pH 7.5, 1 M NaCl). The purified S100A1 was concentrated in a vacuum evaporator and dialyzed against 10 mM HEPES (pH 7.5) and then stored in aliquots at -80°C. Fig. 1a shows the elution profile of the HiTrapQ column and an SDS polyacrylamide gel and Fig. 1B the associated Western Blot which is carried out for quality control of the S100A1 purification. It is seen that the purification procedure used leads to a high degree of purity of the S100A1 protein.

Example 2

Increased intracellular Ca2+ transients in cultivated neonatal rat cardiomyocytes after co-cultivation with recombinant S100A1

Neonatal rat hearts were removed from 3-day-old rats, ventricles separated from the atria and stored coarse-ground on ice in ADS buffer (6.8 g NaCl, 4.76 g HEPES, 0.12 g NaH_2PO_4, 1 g glucose, 0.4 g KCl, 0.1 g $MgSO_4$ and 1000 ml H_2O). In a bioreactor (Wheaton® container), the cells were then separated from the tissue assembly by means of collagenase digestion (108 U/mg/ml collagenase, Worthington, USA), and the released cells separated over a density gradient (Percoll, Pharmacia®) by centrifugation. The cardiomyocyte fraction was resuspended in cell culture medium (DMEM + 10% newborn calf serum) and plated in a density of approx. 100,000 cardiomyocytes per well on 24-well cell culture plates. After 24 hours culture at 37°C and 5% CO_2, recombinant S100A1 was added to the cell culture medium at two-day intervals in concentrations of 1 µM to 10 µM. After 7 days, the measurement of the calcium transients after 1 hour's loading of the cells with FuraPE3AM® took place. The intracellular Ca^{2+} transients were then detected and quantified by means of inverse fluorescence microscopy

(Olympus OSP 3®) under both rest conditions and electrical field stimulation (PHYWE® - bioelectrical measurement unit; frequencies of 30-300 bpm). Fig. 2 shows the original derivations of the analyzed control cells (2a) and of the cells treated with S100A1 (2b). The statistical evaluation shows that under stimulation with S100A1, the rise in the Ca^{2+} concentration per time unit in the systole (+dc/dt) is significantly increased by 3 times ($p<0.0002$) in comparison with the control cell population. The drop in the Ca^{2+} concentration per time unit in the diastole (-dc/dt) is accelerated by 2.2 times ($p<0.0003$) under S100A1 stimulation in comparison with the control cell population. The changes in the Ca^{2+} concentration per time unit were measured as a quotient from the time until the signal maximum and the signal amplitude (+dc/dt) were reached or as a quotient of the time until the half amplitude and the signal amplitude (-dc/dt) were reached. It can be shown with this test approach that S100A1 as a paracrine factor improves the efficiency of the SR function.

Example 3

Preparation of the S100A1 overexpressing virus construct

To prepare the S100A1 overexpressing virus construct, the following strategy is applied: The S100A1 DNA is cloned into the *multi cloning site* behind a CMV promoter into the adenoviral shuttle-vector pAD TRACK (Tong-Chuan He et al. Proc Natl Acad Sci 95; 2509-2514). The resulting plasmid is linearized with PME1 and recombined with pEASY (Tong-Chuan He et al. Proc Natl Acad Sci 95; 2509-2514) in E. coli BJ 5183. The recombination product (pAD/S100A1) corresponds to a plasmid which contains the viral DNA of the E1 and E3 deleted adenovirus type 5, the S100A1 DNA and a kanamycin resistance. Samples of the plasmid pAD/S100A1 were deposited on 26th March 1999 at the DSMZ- Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH [German Collection of Microorganisms and Cell Cultures], Mascheroder Weg 1b, 38124 Brunswick, under the accession number DSM 12755 under the Budapest Treaty. pAD/S100A1 is then transformed in E. coli DH5$_\alpha$ accompanied by kanamycin selection (50 µg/ml) and multiplied. pAD/S100A1 is linearized with PAC1 accompanied by loss of the kanamycin resistance and incorporated by means of lipofectamin (Gibco, BRL) into HEK293 cells for virus production. After 10 days, the

virus is harvested with a low titre and HEK293 infected again. After several cycles of harvesting and reinfecting of HEK293 cells, a high-titre virus finally results which is processed as follows before being introduced into the organism: The HEK293 cells are harvested prior to the cell lysis and centrifuged off (3400 rpm x 10 min). The resulting HEK cell pellet is washed twice in PBS (0.01 M, Sigma, p-3813) and finally taken up in 0.01 M TRIS-pH 8.1. The virus-containing HEK293 cells are then lysed by being frozen four times (liquid nitrogen) and thawed (at 37°C), and the virus released. HEK cell DNA is removed by DNAse digestion (at 37°C over 30 minutes) and HEK cell proteins by freon extraction from the lysate. The virus is purified by means of ultracentrifugation (12 h x 127000 g) through a caesium chloride gradient. The resulting virus fraction is removed and dialyzed for 3 x 2 hours (1 vol-% saccharose in 0.01 M PBS pH 7.4) and stored in aliquots at -80°C. For a gene-therapy treatment, between 10^9 to 10^{12} virus particles are used per gram of heart tissue.

Example 4

Gene therapy of the rabbit heart: Increasing the +dP/dt and the systolic ejection pressure as a result of viral

overexpression of the Ca^{2+}-binding protein S100A1 in the rabbit myocardium.

In the 4th ICR, the thorax of *New Zealand white rabbits* was opened on the right side by lateral thoracotomy. After the aorta was displayed, the opening of the pericardium and the ligation of the aorta took place. By left-cavity injection, an intracoronal perfusion with 2 x 10^{11} virus particles of the recombinant S100A1 virus (n=6), of a virus without S100A1 cDNA (n=11) or of NaCl (n=11) was achieved. 7 days after the operation, the rabbit was catheterized via the a. carotis. The contraction speed (dP/dt) and the systolic ejection pressure (SEP) of the rabbit hearts were measured under basal conditions as well as under isoproterenol stimulation (0.1, 0.5 and 1.0 µg/kg/min). Cryosections were prepared from the rabbit myocardium deep-frozen in liquid nitrogen, and the virus infection of the myocardium demonstrated by fluorescence microscope measurement of the GFP expression (see Fig. 3).

The injection of a virus construct without S100A1 cDNA results in a reduction in the systolic ejection pressure (SEP in mmHg) of the rabbit hearts of 9% on average under all raised conditions in comparison with the animals treated with NaCl. Under basal conditions, the behaviour

of the SEP in the case of the animals treated with S100A1 is statistically not significantly different from both control groups. The SEP in the S100A1 group of the overexpressing rabbits increases by 17% (0.1 µg/kg/min; $p<0.02$), 10% (0.5 µg/kg/min; $p=0.06$) and 11% (1.0 µg/kg/min; $p<0.05$) compared with the group which was treated with the virus construct without S100A1 cDNA under all isoproterenol stimulations. Under isoproterenol stimulation, the animals treated with S100A1 have a SEP which is 4% higher (n.s) compared with the NaCl group (see Fig. 4a).

The contraction speed (dP/dt in mm Hg/s) of the heart falls by an average of 10% upon application of a virus construct without S100A1 cDNA compared with the NaCl injection under all measured conditions which we attribute to a myocarditis. The S100A1 overexpressing animals showed no statistically deviating dP/dt under basal conditions compared with the virus control group. By contrast, the contractility of the heart increased in the S100A1 group under isoproterenol stimulation by 17% (0.1 µg/kg/min; $p<0.05$), 14% (0.5 µg/kg/min; $p<0.03$) and 14% (1.0 µg/kg/min; $p<0.05$) compared with the virus control. The animals treated with the recombinant S100A1 virus showed a dP/dt increased on average by 5% (n.s.)

compared with the NaCl group, despite the myocarditis (see Fig. 4b).

Example 5

Increasing the force transients of *skinned fibres* preparations of the skeletal muscle of the rat through recombinant S100A1 and through peptides of this protein

The binding of Ca^{2+} to S100A1 leads to a modified tertiary structure of this Ca^{2+}-binding protein, resulting in a narrow spatial coordination of the three hydrophobic protein portions (1 amino acids 2-16 [N terminal], cf. SEQ ID NO: 32; 2 amino acids 42-54 [hinge region], cf. SEQ ID NO: 34; 3 amino acids 75-85 [C terminal], cf. SEQ ID NO: 36), which together bind to the RyR (ryanodine receptor is a synonym for Ca^{2+}ATPase of the SR), as data from Treves et al (25) suggest. The aim of this test procedure was therefore to examine what functional significance these sequences - in the form of synthetic peptides - have compared with the whole protein for the regulation of the Ca^{2+} release from the SR. The Ca^{2+} release was measured indirectly on saponin-semipermeabilized skeletal muscle fibres of the rat over an isometric force gradient. The measurement of the

isometric force before and after addition of S100A1 peptide/protein served as a control.

Whereas the individual peptides showed no effect, the combination of "C-terminal" peptide and the "hinge region" increased the isometric force development already by 15%±4%. Both the combination of the three peptides (Fig. 5b) and the recombinant protein (Fig. 5a) in equimolar concentration (5-10 µM) increased the maximum force development in slow skeletal musculature (M. soleus) in the same way by 49%±6% and 52%±7% respectively compared with the control with an unchanged Ca^{2+} sensitivity of the contractile apparatus.

These results show that the effects of S100A1 can be simulated by the hydrophobic protein portions and the full effect of the native protein triggered only by the combination of all three peptides. They thus show the significance of the Ca^{2+}-dependent coordinative regulation of the RyR through the hydrophobic sequences of S100A1.

Legends of the Figures

Fig. 1: (a) Elution profile of HiTrapQ, absorption at 220 nm.

(b) Silver stain after SDS polyacrylamide gel electrophoresis of the individual purification stages: 1: extract from E. coli, 2: proteins not bound by octyl-sepharose, 3: EGTA eluate of octyl sepharose, 4-5: proteins not bound by HiTrapQ, 6-8: fractions of the S100A1 peak of HiTrapQ.

Fig. 2: Original tracings of the analyzed control cells (Fig. 2a) and of the cells treated with S100A1 (Fig. 2b).

Fig. 3: Detection of the viral infection of the myocardium by fluorescence microscope measurement of the GFP expression in cryosections of deep-frozen rabbit myocardium.

Fig. 4: Systolic ejection pressure under isoproterenol stimulation in animals treated with S100A1 compared with the control group (Fig. 4a). Contraction speed under isoproterenol

stimulation in animals treated with S100A1 compared with the control group (Fig. 4b).

Fig. 5: Increasing the maximum force development in slow skeleton musculature (M. soleus) by combining the three S100A1 peptides (N-terminal, hinge region, C-terminal; Fig. 5b) and by recombinant S100A1 protein (Fig. 5a) in equimolar concentration (5-10 µM).

References

(1) Gwathmey JK, Copelas L, MacKinnon R, Schoen FJ, Feldman MD, Grossman W, Morgan JP. Abnormal intracellular calcium handling in myocardium from patients with end-stage heart failure. Circ Res 1987 Jul; 61 (1): 70-76

(2) Beuckelmann DJ, Näbauer M, Erdmann E. Intracellular calcium handling in isolated ventricular myocytes from patients with terminal heart failure. Circulation 1992 Mar; 85 (3): 1046-1055

(3) Hasenfuss G, Holubarsch C, Hermann HP, Astheimer K, Pieske B, Just H. Influence of the force-frequency relationship on haemodynamics and left ventricular function in patients with non-failing hearts and in patients with dilated cardiomyopathy. Eur Heart J 1994 Feb; 15 (2): 164-170

(4) Bohm, Reiger B, Schwinger RH, Erdmann E cAMP concentrations, cAMP dependent protein kinase activity, and phospholamban in non-failing and failing myocardium. Cardiovasc Res 1994 Nov; 28 (11): 1713-9

(5) Packer M, Carver JR, Rodeheffer RJ, Ivanhoe RJ, DiBianco R, Zeldis SM, Hendrix GH, Bommer WJ,

Elkayam U, Kukin ML, et al Effect or oral milrinone on mortality in severe chronic heart failure. The PROMISE Study Research Group. N Engl J Med 1991 Nov 21; 325 (21): 1468-75

(6) Cruickshank JM Phosphodiesterase III inhibitors: long-term risks and short-term benefits. Cardiovasc Drugs Ther 1993 Aug; 7 (4): 655-60

(7) Reddy S, Benatar D, Gheorghiade M Update on digoxin and other oral positive inotropic agents for chronic heart failure. Curr Opin Cardiol 1997 May; 12 (3): 233-41

(8) Zimmermann N, Boknik P, Gams E, Herzig JW, Neumann J, Scholz H Calcium sensitization as new principle of inotropic therapy in end-stage heart failure? Eur J Cardiothorac Surg 1998 Jul; 14 (1): 70-5

(9) Hasenfuss G, Reinecke H, Studer R, Meyer M, Pieske B, Holtz J, Holubarsch C, Posival H, Just H, Drexler H Relation between myocardial function and expression of sarcoplasmic reticulum Ca(2+)-ATPase in failing and nonfailing human myocardium. Circ Res 1994 Sep; 75 (3): 434-442

(10) Meyer M, Schillinger W, Pieske B, Holubarsch C, Heilmann C, Posival H, Kuwajima G, Mikoshiba K,

Just H, Hasenfuss G, et al Alterations of sarcoplasmic reticulum proteins in failing human dilated cardiomyopathy Circulation 1995 Aug 15; 92 (4): 778-784.

(11) Studer R, Reinecke H, Bilger J, Eschenhagen T, Bohm M, Hasenfuss G, Just H, Holtz J, Drexler H. Gene expression of the cardiac Na(+)-Ca2+ exchanger in end-stage human heart failure. Circ Res 1994 Sep; 75 (3): 443-453

(12) Movsesian MA, Karimi M, Green K, Jones LR Ca)2+)-transporting ATPase, phospholamban, and calsequestrin levels in nonfailing and failing human myocardium. Circulation 1994 Aug; 90 (2): 653-657

(13) Schwinger RH, BOHM M, Schmidt U, Karczewski P, Bavendiek U, Flesch M, Krause EG, Erdmann E. Unchanged protein levels of SERCA II and phospholamban but reduced Ca2+ uptake and Ca(2+)-STPase activity of cardiac sarcoplasmic reticulum from dilated cardiomyopathy patients compared with patients with nonfailing hearts. Circulation 1995 Dec 1; 92 (11): 3220-3228

(14) Arai M, Suzuki T, Nagai R. Sarcoplasmic reticulum genes are upregulated in mild cardiac hypertrophy but downregulated in severe cardiac

hypertrophy induced by pressure overload. J Mol Cell Cardiol 1996 Aug; 28 (8): 1583-1590

(15) Schmidt U, Hajjar RJ, Helm PA, Kim CS, Doye AA, Gwathmey JK Contribution of abnormal sarcoplasmic reticulum ATPase activity to systolic and diastolic dysfunction in human heart failure. J Mol Cell Cardiol 1998 Oct; 30 (10): 1929-37

(16) Schäfer BW, Heizmann CW The S100 family of EF-hand calcium-binding proteins: functions and pathology. Trends Biochem Sci 1996 Apr; 21 (4): 134-140

(17) Kato K, Kimura S. S100ao (alpha alpha) protein is mainly located in the heart and striated muscles. Biochim Biophys Acta 1985 Oct 17; 842 (2-3): 146-150

(18) Eschenhagen T et al, FASEB J. 11, 683-694; 1997.

(19) Donato R. Effect of S-100 protein on assembly of brain microtubule proteins in vitro. FEBS Lett 1983 Oct 17; 162 (2): 310-313

(20) Garbuglia M, Verzini M, Giambanco I, Spreca A, Donato R. Effects of calcium-binding proteins (S-100a(o), S-100a, S-100b) on desmin assembly in vitro. FASEB J 1996 Feb; 10 (2): 317-324

(21) Schaper J, Froede R, Hein S, Buck A, Hashizume H, Speiser B, Friedl A, Bleese N. Impairment of

the myocardial ultrastructure and changes of the cytoskeleton in dilated cardiomyopathy. Circulation 1991 Feb; 83 (2): 504-514

(22) Tsutsui H, Ishihara K, Cooper G 4th Cytoskeletal role in the contractile dysfunction of hypertrophied myocardium. Science 1993 Apr 30; 260 (S108): 682-687

(23) Baudier J, Bergeret E, Bertacchi N, Weintraub H, Gagnon J, Garin J Interactions of myogenic bHLH transcription factors with calcium-binding calmodulin and S100a (alpha alpha) proteins. Biochemistry 1995 Jun 20; 34 (24): 7834-7846

(24) Wakasaki H, Koya D, Schoen FJ, Jirousek MR, Ways DK, Hoit BD, Walsh RA, King GL Targeted overexpression of protein kinase C beta2 isoform in myocardium causes cardiomyopathy. Proc Natl Acad Sci USA 1997 Aug 19; 94 (17): 9320-5

(25) Treves S, Scutari E, Robert M, Groh S, Ottolia M, Presipino G, Ronjat M, Zorzato F Interaction of S100A1 with the Ca2+ release channel (ryanodine receptor) of skeletal muscle. Biochemistry 1997 Sep 23; 36 (38): 11496-11503

SEQUENCE LISTING

<110> Hugo A. Katus
Andrew Remppis

<120> Treatment of Cardiac Power Failure

<130> 674-137

<140>
<141> 2000-03-20

<150> DE 199 15 485.6
<151> 1999-04-07

<160> 39

<170> PatentIn Vers. 2.0

<210> 1
<211> 285
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(285)

<223> S100A1 cDNA

<400> 1

```
atg ggc tct gag ctg gag acg gcg atg gag acc ctc atc aac gtg ttc    48
Met Gly Ser Glu Leu Glu Thr Ala Met Glu Thr Leu Ile Asn Val Phe
 1               5                  10                  15

cac gcc cac tcg ggc aaa gag ggg gac aag tac aag ctg agc aag aag    96
His Ala His Ser Gly Lys Glu Gly Asp Lys Tyr Lys Leu Ser Lys Lys
            20                  25                  30

gag ctg aaa gag ctg ctg cag acg gag ctc tct ggc ttc ctg gat gcc   144
Glu Leu Lys Glu Leu Leu Gln Thr Glu Leu Ser Gly Phe Leu Asp Ala
        35                  40                  45

cag aag gat gtg gat gct gtg gac aag gtg atg aag gag cta gac gag   192
Gln Lys Asp Val Asp Ala Val Asp Lys Val Met Lys Glu Leu Asp Glu
    50                  55                  60

aat gga gac ggg gag gtg gac ttc cag gag tat gtg gtg ctt gtg gct   240
Asn Gly Asp Gly Glu Val Asp Phe Gln Glu Tyr Val Val Leu Val Ala
65                  70                  75                  80

gct ctc aca gtg gcc tgt aac aat ttc ttc tgg gag aac agt tga       285
Ala Leu Thr Val Ala Cys Asn Asn Phe Phe Trp Glu Asn Ser
                85                  90                  95
```

<210> 2
<211> 94
<212> PRT
<213> Homo sapiens

<223> S100A1

<400> 2

```
Met Gly Ser Glu Leu Glu Thr Ala Met Glu Thr Leu Ile Asn Val Phe
 1               5                  10                  15
```

His Ala His Ser Gly Lys Glu Gly Asp Lys Tyr Lys Leu Ser Lys Lys
20 25 30

Glu Leu Lys Glu Leu Leu Gln Thr Glu Leu Ser Gly Phe Leu Asp Ala
35 40 45

Gln Lys Asp Val Asp Ala Val Asp Lys Val Met Lys Glu Leu Asp Glu
50 55 60

Asn Gly Asp Gly Glu Val Asp Phe Gln Glu Tyr Val Val Leu Val Ala
65 70 75 80

Ala Leu Thr Val Ala Cys Asn Asn Phe Phe Trp Glu Asn Ser
85 90

<210> 3
<211> 294
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(294)

<223> S100A2 cDNA

<400> 3

atg tgc agt tct ctg gag cag gcg ctg gct gtg ctg gtc act acc ttc 48
Met Cys Ser Ser Leu Glu Gln Ala Leu Ala Val Leu Val Thr Thr Phe
1 5 10 15

cac aag tac tcc tgc caa gag ggc gac aag ttc aag ctg agt aag ggg 96
His Lys Tyr Ser Cys Gln Glu Gly Asp Lys Phe Lys Leu Ser Lys Gly
20 25 30

gaa atg aag gaa ctt ctg cac aag gag ctg ccc agc ttt gtg ggg gag 144
Glu Met Lys Glu Leu Leu His Lys Glu Leu Pro Ser Phe Val Gly Glu
35 40 45

aaa gtg gat gag gag ggg ctg aag aag ctg atg ggc agc ctg gat gag 192
Lys Val Asp Glu Glu Gly Leu Lys Lys Leu Met Gly Ser Leu Asp Glu
50 55 60

aac agt gac cag cag gtg gac ttc cag gag tat gct gtt ttc ctg gca 240
Asn Ser Asp Gln Gln Val Asp Phe Gln Glu Tyr Ala Val Phe Leu Ala
65 70 75 80

ctc atc act gtc atg tgc aat gac ttc ttc cag ggc tgc cca gac cga 288
Leu Ile Thr Val Met Cys Asn Asp Phe Phe Gln Gly Cys Pro Asp Arg
85 90 95

ccc tga 294
Pro

<210> 4
<211> 97
<212> PRT
<213> Homo sapiens

<223> S100A2

<400> 4
Met Cys Ser Ser Leu Glu Gln Ala Leu Ala Val Leu Val Thr Thr Phe
1 5 10 15

```
His Lys Tyr Ser Cys Gln Glu Gly Asp Lys Phe Lys Leu Ser Lys Gly
            20                  25                  30

Glu Met Lys Glu Leu Leu His Lys Glu Leu Pro Ser Phe Val Gly Glu
        35                  40                  45

Lys Val Asp Glu Glu Gly Leu Lys Lys Leu Met Gly Ser Leu Asp Glu
    50                  55                  60

Asn Ser Asp Gln Gln Val Asp Phe Gln Glu Tyr Ala Val Phe Leu Ala
65                  70                  75                  80

Leu Ile Thr Val Met Cys Asn Asp Phe Phe Gln Gly Cys Pro Asp Arg
                85                  90                  95

Pro
```

<210> 5
<211> 306
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(306)

<223> S100A3 cDNA

<400> 5

```
atg gcc agg cct ctg gag cag gcg gta gct gcc atc gtg tgc acc ttc   48
Met Ala Arg Pro Leu Glu Gln Ala Val Ala Ala Ile Val Cys Thr Phe
  1               5                  10                  15

cag gaa tac gca ggg cgc tgt ggg gac aaa tac aag ctc tgc cag gcg   96
Gln Glu Tyr Ala Gly Arg Cys Gly Asp Lys Tyr Lys Leu Cys Gln Ala
            20                  25                  30

gag ctc aag gag ctg ctg cag aag gag ctg gcc acc tgg acc ccg act  144
Glu Leu Lys Glu Leu Leu Gln Lys Glu Leu Ala Thr Trp Thr Pro Thr
        35                  40                  45

gag ttt cgg gaa tgt gac tac aac aaa ttc atg agt gtt ctg gac acc  192
Glu Phe Arg Glu Cys Asp Tyr Asn Lys Phe Met Ser Val Leu Asp Thr
    50                  55                  60

aac aag gac tgc gag gtg gac ttt gtg gag tat gtg cgc tca ctt gcc  240
Asn Lys Asp Cys Glu Val Asp Phe Val Glu Tyr Val Arg Ser Leu Ala
65                  70                  75                  80

tgc ctc tgt ctc tac tgc cac gag tac ttc aag gac tgc ccc tca gag  288
Cys Leu Cys Leu Tyr Cys His Glu Tyr Phe Lys Asp Cys Pro Ser Glu
                85                  90                  95

ccc ccc tgc tcc cag tag                                         306
Pro Pro Cys Ser Gln
            100
```

<210> 6
<211> 101
<212> PRT
<213> Homo sapiens

<223> S100A3

<400> 6

```
Met Ala Arg Pro Leu Glu Gln Ala Val Ala Ala Ile Val Cys Thr Phe
  1               5                  10                  15

Gln Glu Tyr Ala Gly Arg Cys Gly Asp Lys Tyr Lys Leu Cys Gln Ala
             20                  25                  30

Glu Leu Lys Glu Leu Leu Gln Lys Glu Leu Ala Thr Trp Thr Pro Thr
         35                  40                  45

Glu Phe Arg Glu Cys Asp Tyr Asn Lys Phe Met Ser Val Leu Asp Thr
     50                  55                  60

Asn Lys Asp Cys Glu Val Asp Phe Val Glu Tyr Val Arg Ser Leu Ala
 65                  70                  75                  80

Cys Leu Cys Leu Tyr Cys His Glu Tyr Phe Lys Asp Cys Pro Ser Glu
                 85                  90                  95

Pro Pro Cys Ser Gln
            100
```

<210> 7
<211> 306
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(306)

<223> S100A4 cDNA

<400> 7

```
atg gcg tgc cct ctg gag aag gcc ctg gat gtg atg gtg tcc acc ttc   48
Met Ala Cys Pro Leu Glu Lys Ala Leu Asp Val Met Val Ser Thr Phe
  1               5                  10                  15

cac aag tac tcg ggc aaa gag ggt gac aag ttc aag ctc aac aag tca   96
His Lys Tyr Ser Gly Lys Glu Gly Asp Lys Phe Lys Leu Asn Lys Ser
             20                  25                  30

gaa cta aag gag ctg ctg acc cgg gag ctg ccc agc ttc ttg ggg aaa  144
Glu Leu Lys Glu Leu Leu Thr Arg Glu Leu Pro Ser Phe Leu Gly Lys
         35                  40                  45

agg aca gat gaa gct gct ttc cag aag ctg atg agc aac ttg gac agc  192
Arg Thr Asp Glu Ala Ala Phe Gln Lys Leu Met Ser Asn Leu Asp Ser
     50                  55                  60

aac agg gac aac gag gtg gac ttc caa gag tac tgt gtc ttc ctg tcc  240
Asn Arg Asp Asn Glu Val Asp Phe Gln Glu Tyr Cys Val Phe Leu Ser
 65                  70                  75                  80

tgc atc gcc atg atg tgt aac gaa ttc ttt gaa ggc ttc cca gat aag  288
Cys Ile Ala Met Met Cys Asn Glu Phe Phe Glu Gly Phe Pro Asp Lys
                 85                  90                  95

cag ccc agg aag aaa tga                                         306
Gln Pro Arg Lys Lys
            100
```

<210> 8
<211> 101
<212> PRT
<213> Homo sapiens

<223> S100A4

<400> 8

Met Ala Cys Pro Leu Glu Lys Ala Leu Asp Val Met Val Ser Thr Phe
1 5 10 15

His Lys Tyr Ser Gly Lys Glu Gly Asp Lys Phe Lys Leu Asn Lys Ser
20 25 30

Glu Leu Lys Glu Leu Leu Thr Arg Glu Leu Pro Ser Phe Leu Gly Lys
35 40 45

Arg Thr Asp Glu Ala Ala Phe Gln Lys Leu Met Ser Asn Leu Asp Ser
50 55 60

Asn Arg Asp Asn Glu Val Asp Phe Gln Glu Tyr Cys Val Phe Leu Ser
65 70 75 80

Cys Ile Ala Met Met Cys Asn Glu Phe Phe Glu Gly Phe Pro Asp Lys
85 90 95

Gln Pro Arg Lys Lys
100

<210> 9
<211> 333
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(333)

<223> S100A5 cDNA

<400> 9

atg cct gct gct tgg att ctc tgg gct cac tcc cac agt gag ctg cac 48
Met Pro Ala Ala Trp Ile Leu Trp Ala His Ser His Ser Glu Leu His
1 5 10 15

act gtg atg gag act cct ctg gag aag gcc ctg acc act atg gtg acc 96
Thr Val Met Glu Thr Pro Leu Glu Lys Ala Leu Thr Thr Met Val Thr
20 25 30

acg ttt cac aaa tat tcg ggg aga gag ggt agc aaa ctg acc ctg agt 144
Thr Phe His Lys Tyr Ser Gly Arg Glu Gly Ser Lys Leu Thr Leu Ser
35 40 45

agg aag gaa ctc aag gag ctg atc aag aaa gag ctg tgt ctt ggg gag 192
Arg Lys Glu Leu Lys Glu Leu Ile Lys Lys Glu Leu Cys Leu Gly Glu
50 55 60

atg aag gag agc agc atc gat gac ttg atg aag agc ctg gac aag aac 240
Met Lys Glu Ser Ser Ile Asp Asp Leu Met Lys Ser Leu Asp Lys Asn
65 70 75 80

agc gac cag gag atc gac ttc aag gag tac tcg gtg ttc ctg acc atg 288
Ser Asp Gln Glu Ile Asp Phe Lys Glu Tyr Ser Val Phe Leu Thr Met
85 90 95

ctg tgc atg gcc tac aac gac ttc ttt cta gag gac aac aag tga 333
Leu Cys Met Ala Tyr Asn Asp Phe Phe Leu Glu Asp Asn Lys
100 105 110

<210> 10
<211> 110
<212> PRT

<213> Homo sapiens

<223> S100A5

<400> 10

```
Met Pro Ala Ala Trp Ile Leu Trp Ala His Ser His Ser Glu Leu His
  1               5                  10                  15

Thr Val Met Glu Thr Pro Leu Glu Lys Ala Leu Thr Thr Met Val Thr
             20                  25                  30

Thr Phe His Lys Tyr Ser Gly Arg Glu Gly Ser Lys Leu Thr Leu Ser
         35                  40                  45

Arg Lys Glu Leu Lys Glu Leu Ile Lys Lys Glu Leu Cys Leu Gly Glu
     50                  55                  60

Met Lys Glu Ser Ser Ile Asp Asp Leu Met Lys Ser Leu Asp Lys Asn
 65                  70                  75                  80

Ser Asp Gln Glu Ile Asp Phe Lys Glu Tyr Ser Val Phe Leu Thr Met
                 85                  90                  95

Leu Cys Met Ala Tyr Asn Asp Phe Phe Leu Glu Asp Asn Lys
            100                 105                 110
```

<210> 11
<211> 273
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(273)

<223> S100A6 cDNA

<400> 11

```
atg gca tgc ccc ctg gat cag gcc att ggc ctc ctc gtg gcc atc ttc   48
Met Ala Cys Pro Leu Asp Gln Ala Ile Gly Leu Leu Val Ala Ile Phe
  1               5                  10                  15

cac aag tac tcc ggc agg gag ggt gac aag cac acc ctg agc aag aag   96
His Lys Tyr Ser Gly Arg Glu Gly Asp Lys His Thr Leu Ser Lys Lys
             20                  25                  30

gag ctg aag gag ctg atc cag aag gag ctc acc att ggc tcg aag ctg   144
Glu Leu Lys Glu Leu Ile Gln Lys Glu Leu Thr Ile Gly Ser Lys Leu
         35                  40                  45

cag gat gct gaa att gca agg ctg atg gaa gac ttg gac cgg aac aag   192
Gln Asp Ala Glu Ile Ala Arg Leu Met Glu Asp Leu Asp Arg Asn Lys
     50                  55                  60

gac cag gag gtg aac ttc cag gag tat gtc acc ttc ctg ggg gcc ttg   240
Asp Gln Glu Val Asn Phe Gln Glu Tyr Val Thr Phe Leu Gly Ala Leu
 65                  70                  75                  80

gct ttg atc tac aat gaa gcc ctc aag ggc tga                       273
Ala Leu Ile Tyr Asn Glu Ala Leu Lys Gly
                 85                  90
```

<210> 12
<211> 90
<212> PRT
<213> Homo sapiens

<223> S100A6

<400> 12

Met Ala Cys Pro Leu Asp Gln Ala Ile Gly Leu Leu Val Ala Ile Phe
1 5 10 15

His Lys Tyr Ser Gly Arg Glu Gly Asp Lys His Thr Leu Ser Lys Lys
20 25 30

Glu Leu Lys Glu Leu Ile Gln Lys Glu Leu Thr Ile Gly Ser Lys Leu
35 40 45

Gln Asp Ala Glu Ile Ala Arg Leu Met Glu Asp Leu Asp Arg Asn Lys
50 55 60

Asp Gln Glu Val Asn Phe Gln Glu Tyr Val Thr Phe Leu Gly Ala Leu
65 70 75 80

Ala Leu Ile Tyr Asn Glu Ala Leu Lys Gly
85 90

<210> 13
<211> 306
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(306)

<223> S100A7 cDNA

<400> 13

atg agc aac act caa gct gag agg tcc ata ata ggc atg atc gac atg 48
Met Ser Asn Thr Gln Ala Glu Arg Ser Ile Ile Gly Met Ile Asp Met
1 5 10 15

ttt cac aaa tac acc aga cgt gat gac aag att gac aag cca agc ctg 96
Phe His Lys Tyr Thr Arg Arg Asp Asp Lys Ile Asp Lys Pro Ser Leu
20 25 30

ctg acg atg atg aag gag aac ttc ccc aac ttc ctt agt gcc tgt gac 144
Leu Thr Met Met Lys Glu Asn Phe Pro Asn Phe Leu Ser Ala Cys Asp
35 40 45

aaa aag ggc aca aat tac ctc gcc gac gtc ttt gag aaa aag gac aag 192
Lys Lys Gly Thr Asn Tyr Leu Ala Asp Val Phe Glu Lys Lys Asp Lys
50 55 60

aat gag gat aag aag att gat ttt tct gag ttt ctg tcc ttg ctg gga 240
Asn Glu Asp Lys Lys Ile Asp Phe Ser Glu Phe Leu Ser Leu Leu Gly
65 70 75 80

gac ata gcc aca gac tac cac aag cag agc cat gga gca gcg ccc tgt 288
Asp Ile Ala Thr Asp Tyr His Lys Gln Ser His Gly Ala Ala Pro Cys
85 90 95

tcc ggg ggc agc cag tga 306
Ser Gly Gly Ser Gln
100

<210> 14
<211> 101
<212> PRT
<213> Homo sapiens

<223> S100A7

<400> 14

Met Ser Asn Thr Gln Ala Glu Arg Ser Ile Ile Gly Met Ile Asp Met
1 5 10 15

Phe His Lys Tyr Thr Arg Arg Asp Asp Lys Ile Asp Lys Pro Ser Leu
20 25 30

Leu Thr Met Met Lys Glu Asn Phe Pro Asn Phe Leu Ser Ala Cys Asp
35 40 45

Lys Lys Gly Thr Asn Tyr Leu Ala Asp Val Phe Glu Lys Lys Asp Lys
50 55 60

Asn Glu Asp Lys Lys Ile Asp Phe Ser Glu Phe Leu Ser Leu Leu Gly
65 70 75 80

Asp Ile Ala Thr Asp Tyr His Lys Gln Ser His Gly Ala Ala Pro Cys
85 90 95

Ser Gly Gly Ser Gln
100

<210> 15
<211> 282
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(282)

<223> S100A8 cDNA

<400> 15

atg ttg acc gag ctg gag aaa gcc ttg aac tct atc atc gac gtc tac 48
Met Leu Thr Glu Leu Glu Lys Ala Leu Asn Ser Ile Ile Asp Val Tyr
1 5 10 15

cac aag tac tcc ctg ata aag ggg aat ttc cat gcc gtc tac agg gat 96
His Lys Tyr Ser Leu Ile Lys Gly Asn Phe His Ala Val Tyr Arg Asp
20 25 30

gac ctg aag aaa ttg cta gag acc gag tgt cct cag tat atc agg aaa 144
Asp Leu Lys Lys Leu Leu Glu Thr Glu Cys Pro Gln Tyr Ile Arg Lys
35 40 45

aag ggt gca gac gtc tgg ttc aaa gag ttg gat atc aac act gat ggt 192
Lys Gly Ala Asp Val Trp Phe Lys Glu Leu Asp Ile Asn Thr Asp Gly
50 55 60

gca gtt aac ttc cag gag ttc ctc att ctg gtg ata aag atg ggc gtg 240
Ala Val Asn Phe Gln Glu Phe Leu Ile Leu Val Ile Lys Met Gly Val
65 70 75 80

gca gcc cac aaa aaa agc cat gaa gaa agc cac aaa gag tag 282
Ala Ala His Lys Lys Ser His Glu Glu Ser His Lys Glu
85 90

<210> 16
<211> 93
<212> PRT
<213> Homo sapiens

<223> S100A8

<400> 16
Met Leu Thr Glu Leu Glu Lys Ala Leu Asn Ser Ile Ile Asp Val Tyr
1 5 10 15

His Lys Tyr Ser Leu Ile Lys Gly Asn Phe His Ala Val Tyr Arg Asp
20 25 30

Asp Leu Lys Lys Leu Leu Glu Thr Glu Cys Pro Gln Tyr Ile Arg Lys
35 40 45

Lys Gly Ala Asp Val Trp Phe Lys Glu Leu Asp Ile Asn Thr Asp Gly
50 55 60

Ala Val Asn Phe Gln Glu Phe Leu Ile Leu Val Ile Lys Met Gly Val
65 70 75 80

Ala Ala His Lys Lys Ser His Glu Glu Ser His Lys Glu
85 90

<210> 17
<211> 345
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(345)

<223> S100A9 cDNA

<400> 17

```
atg act tgc aaa atg tcg cag ctg gaa cgc aac ata gag acc atc atc   48
Met Thr Cys Lys Met Ser Gln Leu Glu Arg Asn Ile Glu Thr Ile Ile
 1               5                  10                  15

aac acc ttc cac caa tac tct gtg aag ctg ggg cac cca gac acc ctg   96
Asn Thr Phe His Gln Tyr Ser Val Lys Leu Gly His Pro Asp Thr Leu
            20                  25                  30

aac cag ggg gaa ttc aaa gag ctg gtg cga aaa gat ctg caa aat ttt  144
Asn Gln Gly Glu Phe Lys Glu Leu Val Arg Lys Asp Leu Gln Asn Phe
        35                  40                  45

ctc aag aag gag aat aag aat gaa aag gtc ata gaa cac atc atg gag  192
Leu Lys Lys Glu Asn Lys Asn Glu Lys Val Ile Glu His Ile Met Glu
    50                  55                  60

gac ctg gac aca aat gca gac aag cag ctg agc ttc gag gag ttc atc  240
Asp Leu Asp Thr Asn Ala Asp Lys Gln Leu Ser Phe Glu Glu Phe Ile
65                  70                  75                  80

atg ctg atg gcg agg cta acc tgg gcc tcc cac gag aag atg cac gag  288
Met Leu Met Ala Arg Leu Thr Trp Ala Ser His Glu Lys Met His Glu
                85                  90                  95

ggt gac gag ggc cct ggc cac cac cat aag cca ggc ctc ggg gag ggc  336
Gly Asp Glu Gly Pro Gly His His His Lys Pro Gly Leu Gly Glu Gly
            100                 105                 110

acc ccc taa                                                     345
Thr Pro
        115
```

<210> 18
<211> 114

<212> PRT
<213> Homo sapiens

<223> S100A9

<400> 18

Met Thr Cys Lys Met Ser Gln Leu Glu Arg Asn Ile Glu Thr Ile Ile
1 5 10 15

Asn Thr Phe His Gln Tyr Ser Val Lys Leu Gly His Pro Asp Thr Leu
20 25 30

Asn Gln Gly Glu Phe Lys Glu Leu Val Arg Lys Asp Leu Gln Asn Phe
35 40 45

Leu Lys Lys Glu Asn Lys Asn Glu Lys Val Ile Glu His Ile Met Glu
50 55 60

Asp Leu Asp Thr Asn Ala Asp Lys Gln Leu Ser Phe Glu Glu Phe Ile
65 70 75 80

Met Leu Met Ala Arg Leu Thr Trp Ala Ser His Glu Lys Met His Glu
85 90 95

Gly Asp Glu Gly Pro Gly His His His Lys Pro Gly Leu Gly Glu Gly
100 105 110

Thr Pro

<210> 19
<211> 294
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(294)

<223> S100A10 cDNA

<400> 19

atg cca tct caa atg gaa cac gcc atg gaa acc atg atg ttt aca ttt 48
Met Pro Ser Gln Met Glu His Ala Met Glu Thr Met Met Phe Thr Phe
1 5 10 15

cac aaa ttc gct ggg gat aaa ggc tac tta aca aag gag gac ctg aga 96
His Lys Phe Ala Gly Asp Lys Gly Tyr Leu Thr Lys Glu Asp Leu Arg
20 25 30

gta ctc atg gaa aag gag ttc cct gga ttt ttg gaa aat caa aaa gac 144
Val Leu Met Glu Lys Glu Phe Pro Gly Phe Leu Glu Asn Gln Lys Asp
35 40 45

cct ctg gct gtg gac aaa ata atg aag gac ctg gac cag tgt aga gat 192
Pro Leu Ala Val Asp Lys Ile Met Lys Asp Leu Asp Gln Cys Arg Asp
50 55 60

ggc aaa gtg ggc ttc cag agc ttc ttt tcc cta att gcg ggc ctc acc 240
Gly Lys Val Gly Phe Gln Ser Phe Phe Ser Leu Ile Ala Gly Leu Thr
65 70 75 80

att gca tgc aat gac tat ttt gta gta cac atg aag cag aag gga aag 288
Ile Ala Cys Asn Asp Tyr Phe Val Val His Met Lys Gln Lys Gly Lys
85 90 95

aag tag 294
Lys

<210> 20
<211> 97
<212> PRT
<213> Homo sapiens

<223> S100A10

<400> 20

Met Pro Ser Gln Met Glu His Ala Met Glu Thr Met Met Phe Thr Phe
1 5 10 15

His Lys Phe Ala Gly Asp Lys Gly Tyr Leu Thr Lys Glu Asp Leu Arg
20 25 30

Val Leu Met Glu Lys Glu Phe Pro Gly Phe Leu Glu Asn Gln Lys Asp
35 40 45

Pro Leu Ala Val Asp Lys Ile Met Lys Asp Leu Asp Gln Cys Arg Asp
50 55 60

Gly Lys Val Gly Phe Gln Ser Phe Phe Ser Leu Ile Ala Gly Leu Thr
65 70 75 80

Ile Ala Cys Asn Asp Tyr Phe Val Val His Met Lys Gln Lys Gly Lys
85 90 95

Lys

<210> 21
<211> 318
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(318)

<223> S100A11 cDNA

<400> 21

atg gca aaa atc tcc agc cct aca gag act gag cgg tgc atc gag tcc 48
Met Ala Lys Ile Ser Ser Pro Thr Glu Thr Glu Arg Cys Ile Glu Ser
1 5 10 15

ctg att gct gtc ttc cag aag tat gct gga aag gat ggt tat aac tac 96
Leu Ile Ala Val Phe Gln Lys Tyr Ala Gly Lys Asp Gly Tyr Asn Tyr
20 25 30

act ctc tcc aag aca gag ttc cta agc ttc atg aat aca gaa cta gct 144
Thr Leu Ser Lys Thr Glu Phe Leu Ser Phe Met Asn Thr Glu Leu Ala
35 40 45

gcc ttc aca aag aac cag aag gac cct ggt gtc ctt gac cgc atg atg 192
Ala Phe Thr Lys Asn Gln Lys Asp Pro Gly Val Leu Asp Arg Met Met
50 55 60

aag aaa ctg gac acc aac agt gat ggt cag cta gat ttc tca gaa ttt 240
Lys Lys Leu Asp Thr Asn Ser Asp Gly Gln Leu Asp Phe Ser Glu Phe
65 70 75 80

ctt aat ctg att ggt ggc cta gct atg gct tgc cat gac tcc ttc ctc 288
Leu Asn Leu Ile Gly Gly Leu Ala Met Ala Cys His Asp Ser Phe Leu
85 90 95

aag gct gtc cct tcc cag aag cgg acc tga 318
Lys Ala Val Pro Ser Gln Lys Arg Thr
100 105

<210> 22
<211> 105
<212> PRT
<213> Homo sapiens

<223> S100A11

<400> 22

```
Met Ala Lys Ile Ser Ser Pro Thr Glu Thr Glu Arg Cys Ile Glu Ser
  1               5                  10                  15

Leu Ile Ala Val Phe Gln Lys Tyr Ala Gly Lys Asp Gly Tyr Asn Tyr
            20                  25                  30

Thr Leu Ser Lys Thr Glu Phe Leu Ser Phe Met Asn Thr Glu Leu Ala
        35                  40                  45

Ala Phe Thr Lys Asn Gln Lys Asp Pro Gly Val Leu Asp Arg Met Met
    50                  55                  60

Lys Lys Leu Asp Thr Asn Ser Asp Gly Gln Leu Asp Phe Ser Glu Phe
65                  70                  75                  80

Leu Asn Leu Ile Gly Gly Leu Ala Met Ala Cys His Asp Ser Phe Leu
                85                  90                  95

Lys Ala Val Pro Ser Gln Lys Arg Thr
            100                 105
```

<210> 23
<211> 279
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(279)

<223> S100A12 cDNA

<400> 23

```
atg aca aaa ctt gaa gag cat ctg gag gga att gtc aat atc ttc cac   48
Met Thr Lys Leu Glu Glu His Leu Glu Gly Ile Val Asn Ile Phe His
  1               5                  10                  15

caa tac tca gtt cgg aag ggg cat ttt gac acc ctc tct aag ggt gag   96
Gln Tyr Ser Val Arg Lys Gly His Phe Asp Thr Leu Ser Lys Gly Glu
            20                  25                  30

ctg aag cag ctg ctt aca aag gag ctt gca aac acc atc aag aat atc   144
Leu Lys Gln Leu Leu Thr Lys Glu Leu Ala Asn Thr Ile Lys Asn Ile
        35                  40                  45
```

```
aaa gat aaa gct gtc att gat gaa ata ttc caa ggc ctg gat gct aat   192
Lys Asp Lys Ala Val Ile Asp Glu Ile Phe Gln Gly Leu Asp Ala Asn
    50                  55                  60

caa gat gaa cag gtc gac ttt caa gaa ttc ata tcc ctg gta gcc att   240
Gln Asp Glu Gln Val Asp Phe Gln Glu Phe Ile Ser Leu Val Ala Ile
65                  70                  75                  80

gcg ctg aag gct gcc cat tac cac acc cac aaa gag tag               279
Ala Leu Lys Ala Ala His Tyr His Thr His Lys Glu
                85                  90
```

<210> 24

<211> 92
<212> PRT
<213> Homo sapiens

<223> S100A12

<400> 24
Met Thr Lys Leu Glu Glu His Leu Glu Gly Ile Val Asn Ile Phe His
1 5 10 15

Gln Tyr Ser Val Arg Lys Gly His Phe Asp Thr Leu Ser Lys Gly Glu
20 25 30

Leu Lys Gln Leu Leu Thr Lys Glu Leu Ala Asn Thr Ile Lys Asn Ile
35 40 45

Lys Asp Lys Ala Val Ile Asp Glu Ile Phe Gln Gly Leu Asp Ala Asn
50 55 60

Gln Asp Glu Gln Val Asp Phe Gln Glu Phe Ile Ser Leu Val Ala Ile
65 70 75 80

Ala Leu Lys Ala Ala His Tyr His Thr His Lys Glu
85 90

<210> 25
<211> 297
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(297)
<223> S100A13 cDNA

<400> 25

atg gca gca gaa cca ctg aca gag cta gag gag tcc att gag acc gtg 48
Met Ala Ala Glu Pro Leu Thr Glu Leu Glu Glu Ser Ile Glu Thr Val
1 5 10 15

gtc acc acc ttc ttc acc ttt gca agg cag gag ggc cgg aag gat agc 96
Val Thr Thr Phe Phe Thr Phe Ala Arg Gln Glu Gly Arg Lys Asp Ser
20 25 30

ctc agc gtc aac gag ttc aaa gag ctg gtt acc cag cag ttg ccc cat 144
Leu Ser Val Asn Glu Phe Lys Glu Leu Val Thr Gln Gln Leu Pro His
35 40 45

ctg ctc aag gat gtg ggc tct ctt gat gag aag atg aag agc ttg gat 192
Leu Leu Lys Asp Val Gly Ser Leu Asp Glu Lys Met Lys Ser Leu Asp
50 55 60

gtg aat cag gac tcg gag ctc aag ttc aat gag tac tgg aga ttg att 240
Val Asn Gln Asp Ser Glu Leu Lys Phe Asn Glu Tyr Trp Arg Leu Ile
65 70 75 80

ggg gag ctg gcc aag gaa atc agg aag aag aaa gac ctg aag atc agg 288
Gly Glu Leu Ala Lys Glu Ile Arg Lys Lys Lys Asp Leu Lys Ile Arg
85 90 95

aag aag taa 297
Lys Lys

<210> 26
<211> 98
<212> PRT

<213> Homo sapiens

<223> S100A13

<400> 26

```
Met Ala Ala Glu Pro Leu Thr Glu Leu Glu Glu Ser Ile Glu Thr Val
  1               5                  10                  15

Val Thr Thr Phe Phe Thr Phe Ala Arg Gln Glu Gly Arg Lys Asp Ser
             20                  25                  30

Leu Ser Val Asn Glu Phe Lys Glu Leu Val Thr Gln Gln Leu Pro His
         35                  40                  45

Leu Leu Lys Asp Val Gly Ser Leu Asp Glu Lys Met Lys Ser Leu Asp
     50                  55                  60

Val Asn Gln Asp Ser Glu Leu Lys Phe Asn Glu Tyr Trp Arg Leu Ile
 65                  70                  75                  80

Gly Glu Leu Ala Lys Glu Ile Arg Lys Lys Lys Asp Leu Lys Ile Arg
                 85                  90                  95

Lys Lys
```

<210> 27
<211> 288
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(288)

<223> S100B cDNA

<400> 27

```
atg acg gaa cta gag aca gcc atg ggc atg atc ata gac gtc ttt tcc   48
Met Thr Glu Leu Glu Thr Ala Met Gly Met Ile Ile Asp Val Phe Ser
  1               5                  10                  15

cga tat tcg ggc agc gag ggc agc acg cag acc ctg acc aag ggg gag   96
Arg Tyr Ser Gly Ser Glu Gly Ser Thr Gln Thr Leu Thr Lys Gly Glu
             20                  25                  30

ctc aag gtg ctg atg gag aag gag cta cca ggc ttc ctg cag agt gga   144
Leu Lys Val Leu Met Glu Lys Glu Leu Pro Gly Phe Leu Gln Ser Gly
         35                  40                  45

aaa gac aag gat gcc gtg gat aaa ttg ctc aag gac ctg gac gcc aat   192
Lys Asp Lys Asp Ala Val Asp Lys Leu Leu Lys Asp Leu Asp Ala Asn
     50                  55                  60

gga gat gcc cag gtg gac ttc agt gag ttc atc gtg ttc gtg gct gca   240
Gly Asp Ala Gln Val Asp Phe Ser Glu Phe Ile Val Phe Val Ala Ala
 65                  70                  75                  80

atc acg tct gcc tgt cac aag tac ttt gag aag gca gga ctc aaa tga   288
Ile Thr Ser Ala Cys His Lys Tyr Phe Glu Lys Ala Gly Leu Lys
                 85                  90                  95
```

<210> 28
<211> 95
<212> PRT
<213> Homo sapiens

<223> S100B

<400> 28

Met Thr Glu Leu Glu Thr Ala Met Gly Met Ile Ile Asp Val Phe Ser
1 5 10 15

Arg Tyr Ser Gly Ser Glu Gly Ser Thr Gln Thr Leu Thr Lys Gly Glu
20 25 30

Leu Lys Val Leu Met Glu Lys Glu Leu Pro Gly Phe Leu Gln Ser Gly
35 40 45

Lys Asp Lys Asp Ala Val Asp Lys Leu Leu Lys Asp Leu Asp Ala Asn
50 55 60

Gly Asp Ala Gln Val Asp Phe Ser Glu Phe Ile Val Phe Val Ala Ala
65 70 75 80

Ile Thr Ser Ala Cys His Lys Tyr Phe Glu Lys Ala Gly Leu Lys
85 90 95

<210> 29
<211> 288
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(288)

<223> S100P cDNA

<400> 29

```
atg acg gaa cta gag aca gcc atg ggc atg atc ata gac gtc ttt tcc   48
Met Thr Glu Leu Glu Thr Ala Met Gly Met Ile Ile Asp Val Phe Ser
 1               5                  10                  15

cga tat tcg ggc agc gag ggc agc acg cag acc ctg acc aag ggg gag   96
Arg Tyr Ser Gly Ser Glu Gly Ser Thr Gln Thr Leu Thr Lys Gly Glu
            20                  25                  30

ctc aag gtg ctg atg gag aag gag cta cca ggc ttc ctg cag agt gga   144
Leu Lys Val Leu Met Glu Lys Glu Leu Pro Gly Phe Leu Gln Ser Gly
        35                  40                  45

aaa gac aag gat gcc gtg gat aaa ttg ctc aag gac ctg gac gcc aat   192
Lys Asp Lys Asp Ala Val Asp Lys Leu Leu Lys Asp Leu Asp Ala Asn
    50                  55                  60

gga gat gcc cag gtg gac ttc agt gag ttc atc gtg ttc gtg gct gca   240
Gly Asp Ala Gln Val Asp Phe Ser Glu Phe Ile Val Phe Val Ala Ala
65                  70                  75                  80

atc acg tct gcc tgt cac aag tac ttt gag aag gca gga ctc aaa tga   288
Ile Thr Ser Ala Cys His Lys Tyr Phe Glu Lys Ala Gly Leu Lys
                85                  90                  95
```

<210> 30
<211> 95
<212> PRT
<213> Homo sapiens

<223> S100P

<400> 30
Met Thr Glu Leu Glu Thr Ala Met Gly Met Ile Ile Asp Val Phe Ser
1 5 10 15

Arg Tyr Ser Gly Ser Glu Gly Ser Thr Gln Thr Leu Thr Lys Gly Glu
20 25 30

Leu Lys Val Leu Met Glu Lys Glu Leu Pro Gly Phe Leu Gln Ser Gly
35 40 45

Lys Asp Lys Asp Ala Val Asp Lys Leu Leu Lys Asp Leu Asp Ala Asn
50 55 60

Gly Asp Ala Gln Val Asp Phe Ser Glu Phe Ile Val Phe Val Ala Ala
65 70 75 80

Ile Thr Ser Ala Cys His Lys Tyr Phe Glu Lys Ala Gly Leu Lys
85 90 95

<210> 31
<211> 45
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(45)

<223> S100A1 cDNA [N-terminal]

<400> 31
ggc tct gag ctg gag acg gcg atg gag acc ctc atc aac gtg ttc 45
Gly Ser Glu Leu Glu Thr Ala Met Glu Thr Leu Ile Asn Val Phe
1 5 10 15

<210> 32
<211> 15
<212> PRT
<213> Homo sapiens

<223> S100A1 [N-terminal]

<400> 32
Gly Ser Glu Leu Glu Thr Ala Met Glu Thr Leu Ile Asn Val Phe
1 5 10 15

<210> 33
<211> 39
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(39)

<223> S100A1 cDNA [hinge-region]

<400> 33
ctc tct ggc ttc ctg gat gcc cag aag gat gtg gat gct 39
Leu Ser Gly Phe Leu Asp Ala Gln Lys Asp Val Asp Ala
1 5 10

<210> 34
<211> 13
<212> PRT
<213> Homo sapiens

<223> S100A1 [hinge-region]

<400> 34
Leu Ser Gly Phe Leu Asp Ala Gln Lys Asp Val Asp Ala
1 5 10

<210> 35
<211> 33
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(33)
<223> S100A1 cDNA [C-terminal]

<400> 35
tat gtg gtg ctt gtg gct gct ctc aca gtg gcc 33
Tyr Val Val Leu Val Ala Ala Leu Thr Val Ala
1 5 10

<210> 36
<211> 11
<212> PRT
<213> Homo sapiens

<223> S100A1 [C-terminal]

<400> 36
Tyr Val Val Leu Val Ala Ala Leu Thr Val Ala
1 5 10

<210> 37
<211> 17
<212> PRT
<213> Homo sapiens

<223> S100A1 [N-terminal, modified]

<400> 37
Gly Ser Glu Leu Glu Thr Ala Met Glu Thr Leu Ile Asn Val Phe His
1 5 10 15

Ala

<210> 38
<211> 13
<212> PRT
<213> Homo sapiens

<223> S100A1 [hinge-region, modified]

<400> 38
Leu Ser Gly Phe Leu Asp Ala Gln Lys Asp Ala Asp Ala
1 5 10

<210> 39
<211> 17
<212> PRT
<213> Homo sapiens

<223> S100A1 [C-terminal, modified]

<400> 39
Asp Lys Asp Asp Pro Pro Tyr Val Val Leu Val Ala Ala Leu Thr Val
1 5 10 15

Ala

Claims

1. A use of a medicament for the treatment of heart diseases selected from the group consisting of primary cardiomyopathies and secondary cardiomyopathies, wherein the medicament comprises a nucleic acid molecule comprising sequence(s) coding for one or more S100 proteins selected from the group consisting of S100A1 as shown in SEQ ID NO:2, S100A2 as shown in SEQ ID NO:4, S100A3 as shown in SEQ ID NO:6, S100A4 as shown in SEQ ID NO:8, S100A5 as shown in SEQ ID NO:10, S100A6 as shown in SEQ ID NO:12, S100A7 as shown in SEQ ID NO:14, S100A8 as shown in SEQ ID NO:16, S100A9 as shown in SEQ ID NO:18, S100A10 as shown in SEQ ID NO:20, S100A11 as shown in SEQ ID NO:22, S100A12 as shown in SEQ ID NO:24, S100A13 as shown in SEQ ID NO:26 and S100β as shown in SEQ ID NO:28, one or more mutants of the same having an identity of at least 60% thereto which increase the systolic Ca^{2+} release from the sarcoplasmic reticulum and increase the rate of Ca^{2+} uptake into the sarcoplasmic reticulum, or several hydrophobic fragments of the same, which increase the systolic Ca^{2+} release from the sarcoplasmic reticulum and increase the rate of Ca^{2+} uptake into the sarcoplasmic reticulum.

2. A use of a medicament for the treatment of heart diseases selected from the group consisting of primary cardiomyopathies and secondary cardiomyopathies, wherein the medicament contains a gene transfer vector which contains nucleic acid sequence(s) coding for one or more S100 proteins selected from the group consisting of S100A1 as shown in SEQ ID NO:2, S100A2 as shown in SEQ ID NO:4, S100A3 as shown in SEQ ID NO:6, S100A4 as shown in SEQ ID NO:8, S100A5 as shown in SEQ ID NO:10, S100A6 as shown in SEQ ID NO:12, S100A7 as shown in SEQ ID NO:14, S100A8 as shown in SEQ ID NO:16, S100A9 as shown in SEQ ID NO:18, S100A10 as shown in SEQ ID NO:20, S100A11 as shown in SEQ ID NO:22, S100A12 as shown in SEQ ID NO:24, S100A13 as shown in SEQ ID NO:26 and S100β as shown in SEQ ID NO:28, one or more mutants of the same

having an identity of at least 60% thereto which increase the systolic Ca^{2+} release from the sarcoplasmic reticulum and increase the rate of Ca^{2+} uptake into the sarcoplasmic reticulum, or several hydrophobic fragments of the same, which increase the systolic Ca^{2+} release from the sarcoplasmic reticulum and increase the rate of Ca^{2+} uptake into the sarcoplasmic reticulum.

3. The use according to claim 1 or 2, wherein the primary cardiomyopathies comprise hereditary cardiomyopathies or cardiomyopathies due to spontaneous mutations.

4. The use according to claim 1 or 2, wherein the secondary cardiomyopathies comprise ischaemic cardiomyopathy due to an arteriosclerosis, dilative cardiomyopathy due to an infectious or toxic disease of the myocardium, hypertensive heart disease due to a pulmonary-arterial or an arterial hypertonia, structural heart diseases due to rhythm disturbances or diseases of the heart valves.

5. A use of a medicament for the treatment of cardiac insufficiency, wherein the medicament contains a nucleic acid molecule comprising nucleic acid sequence(s) coding for one or more S100 proteins selected from the group consisting of S100A1 as shown in SEQ ID NO:2, S100A2 as shown in SEQ ID NO:4, S100A3 as shown in SEQ ID NO:6, S100A4 as shown in SEQ ID NO:8, S100A5 as shown in SEQ ID NO:10, S100A6 as shown in SEQ ID NO:12, S100A7 as shown in SEQ ID NO:14, S100A8 as shown in SEQ ID NO:16, S100A9 as shown in SEQ ID NO:18, S100A10 as shown in SEQ ID NO:20, S100A11 as shown in SEQ ID NO:22, S100A12 as shown in SEQ ID NO:24, S100A13 as shown in SEQ ID NO:26 and S100β as shown in SEQ ID NO:28, one or more mutants of the same having an identity of at least 60% thereto which increase the systolic Ca^{2+} release from the sarcoplasmic reticulum and increase the rate of Ca^{2+} uptake into the sarcoplasmic reticulum, or several hydrophobic fragments of the same, which increase the systolic Ca^{2+} release from the sarcoplasmic reticulum and increase the rate of Ca^{2+} uptake into the sarcoplasmic reticulum.

6. A use of a medicament for the treatment of cardiac insufficiency, wherein the medicament contains a gene transfer vector which contains nucleic acid sequence(s) coding for one or more S100 proteins selected from the group consisting of S100A1 as shown in SEQ ID NO:2, S100A2 as shown in SEQ ID NO:4, S100A3 as shown in SEQ ID NO:6, S100A4 as shown in SEQ ID NO:8, S100A5 as shown in SEQ ID NO:10, S100A6 as shown in SEQ ID NO:12, S100A7 as shown in SEQ ID NO:14, S100A8 as shown in SEQ ID NO:16, S100A9 as shown in SEQ ID NO:18, S100A10 as shown in SEQ ID NO:20, S100A11 as shown in SEQ ID NO:22, S100A12 as shown in SEQ ID NO:24, S100A13 as shown in SEQ ID NO:26 and S100β as shown in SEQ ID NO:28, one or more mutants of the same having an identity of at least 60% thereto which increase the systolic Ca^{2+} release from the sarcoplasmic reticulum and increase the rate of Ca^{2+} uptake into the sarcoplasmic reticulum, or several hydrophobic fragments of the same, which increase the systolic Ca^{2+} release from the sarcoplasmic reticulum and increase the rate of Ca^{2+} uptake into the sarcoplasmic reticulum.

7. The use according to any one of claims 1-6, wherein the S100 protein comprises S100A1 protein comprising the amino acid sequence displayed in SEQ ID NO: 2.

8. The use according to any one of claims 1-6, wherein the fragments are selected from the group consisting of the amino acid sequences shown in SEQ ID NO: 32, 34 and 36.

9. The use according to any one of claims 1 to 6, wherein the medicament comprises a combination of at least two of the fragments represented in SEQ ID NO: 32, 34, 36, 37, 38 and 39.

10. The use according to any one of claims 1 to 6, wherein the medicament contains a nucleic acid sequence coding for S100A1 according to SEQ ID NO: 1 or a gene transfer vector containing this sequence.

11. The use according to any one of claims 1 to 6, wherein the nucleic acid sequences coding for the fragments are selected from the group consisting of SEQ ID NO: 31, 33, 35 and from the nucleic acid sequences coding for the fragments according to SEQ ID NO: 37, 38 or 39 or are contained in the form of gene transfer vector(s) containing these sequences.

12. The use according to any one of claims 1 to 6, wherein the medicament contains a combination of at least two of the nucleic acid sequences coding for SEQ ID NO: 32, 34, 36, 37, 38 and 39, or at least two of the nucleic acid sequences represented in SEQ ID NO: 31, 33, 35 and sequences coding for SEQ ID NO: 37, 38 and 39, or one or more gene transfer vectors containing this combination.

13. The use according to any one of claims 1 to 12, wherein the medicament is formulated for oral, intravenous, intraarterial or intracoronal application.

14. The use according to any one of claims 1 to 13, wherein the medicament is formulated with pharmaceutically compatible auxiliaries or supports.

15. A use of one or more S100 proteins for the preparation of a medicament for the treatment of heart diseases selected from the group consisting of primary cardiomyopathies and secondary cardiomyopathies, wherein the S100 proteins are selected from the group consisting of S100A1 as shown in SEQ ID NO:2, S100A2 as shown in SEQ ID NO:4, S100A3 as shown in SEQ ID NO:6, S100A4 as shown in SEQ ID NO:8, S100A5 as shown in SEQ ID NO:10, S100A6 as shown in SEQ ID NO:12, S100A7 as shown in SEQ ID NO:14, S100A8 as shown in SEQ ID NO:16, S100A9 as shown in SEQ ID NO:18, S100A10 as shown in SEQ ID NO:20, S100A11 as shown in SEQ ID NO:22, S100A12 as shown in SEQ ID NO:24, S100A13 as shown in SEQ ID NO:26 and S100β as shown in SEQ ID NO:28, one or more mutants of the same having an identity of at least 60% thereto which increase the systolic Ca^{2+} release from the sarcoplasmic reticulum and increase the rate of Ca^{2+} uptake into the sarcoplasmic reticulum, or several hydrophobic fragments of the same, which increase the systolic Ca^{2+} release from the sarcoplasmic

reticulum and increase the rate of Ca^{2+} uptake into the sarcoplasmic reticulum.

16. A use of one or more nucleic acid molecules comprising nucleic acid sequences which code for one or more S100 proteins, for the preparation of a medicament for the treatment of heart diseases selected from the group consisting of primary cardiomyopathies and secondary cardiomyopathies, wherein the one or more S100 proteins are selected from the group consisting of S100A1 as shown in SEQ ID NO:2, S100A2 as shown in SEQ ID NO:4, S100A3 as shown in SEQ ID NO:6, S100A4 as shown in SEQ ID NO:8, S100A5 as shown in SEQ ID NO:10, S100A6 as shown in SEQ ID NO:12, S100A7 as shown in SEQ ID NO:14, S100A8 as shown in SEQ ID NO:16, S100A9 as shown in SEQ ID NO:18, S100A10 as shown in SEQ ID NO:20, S100A11 as shown in SEQ ID NO:22, S100A12 as shown in SEQ ID NO:24, S100A13 as shown in SEQ ID NO:26 and S100β as shown in SEQ ID NO:28, one or more mutants of the same having an identity of at least 60% thereto which increase the systolic Ca^{2+} release from the sarcoplasmic reticulum and increase the rate of Ca^{2+} uptake into the sarcoplasmic reticulum, or several hydrophobic fragments of the same, which increase the systolic Ca^{2+} release from the sarcoplasmic reticulum and increase the rate of Ca^{2+} uptake into the sarcoplasmic reticulum.

17. A use of a gene transfer vector which contains nucleic acid sequence(s) coding for one or more S100 proteins for the preparation of a medicament for the treatment of heart diseases from the group composed of primary cardiomyopathies and secondary cardiomyopathies, wherein the one or more S100 proteins are selected from the group consisting of S100A1 as shown in SEQ ID NO:2, S100A2 as shown in SEQ ID NO:4, S100A3 as shown in SEQ ID NO:6, S100A4 as shown in SEQ ID NO:8, S100A5 as shown in SEQ ID NO:10, S100A6 as shown in SEQ ID NO:12, S100A7 as shown in SEQ ID NO:14, S100A8 as shown in SEQ ID NO:16, S100A9 as shown in SEQ ID NO:18, S100A10 as shown in SEQ ID NO:20, S100A11 as shown in SEQ ID NO:22, S100A12 as shown in SEQ ID NO:24, S100A13 as shown in SEQ ID

NO:26 and S100β as shown in SEQ ID NO:28, one or more mutants of the same having an identity of at least 60% thereto which increase the systolic Ca^{2+} release from the sarcoplasmic reticulum and increase the rate of Ca^{2+} uptake into the sarcoplasmic reticulum, or several hydrophobic fragments of the same, which increase the systolic Ca^{2+} release from the sarcoplasmic reticulum and increase the rate of Ca^{2+} uptake into the sarcoplasmic reticulum.

18. The use according to any one of claims 15 to 17, wherein the primary cardiomyopathies comprise hereditary cardiomyopathies or cardiomyopathies due to spontaneous mutations.

19. The use according to any one of claims 15 to 17, wherein the secondary cardiomyopathies include ischaemic cardiomyopathy due to an arteriosclerosis, dilatative cardiomyopathy due to an infectious or toxic disease of the myocardium, hypertensive heart disease due to a pulmonary-arterial or an arterial hypertonia, structural heart diseases due to rhythm disturbances or diseases of the heart valves.

20. A use of one or more S100 proteins, for the preparation of a medicament for the treatment of cardiac insufficiency, wherein the one or more S100 proteins are selected from the group consisting of S100A1 as shown in SEQ ID NO:2, S100A2 as shown in SEQ ID NO:4, S100A3 as shown in SEQ ID NO:6, S100A4 as shown in SEQ ID NO:8, S100A5 as shown in SEQ ID NO:10, S100A6 as shown in SEQ ID NO:12, S100A7 as shown in SEQ ID NO:14, S100A8 as shown in SEQ ID NO:16, S100A9 as shown in SEQ ID NO:18, S100A10 as shown in SEQ ID NO:20, S100A11 as shown in SEQ ID NO:22, S100A12 as shown in SEQ ID NO:24, S100A13 as shown in SEQ ID NO:26 and S100β as shown in SEQ ID NO:28, one or more mutants of the same having an identity of at least 60% thereto which increase the systolic Ca^{2+} release from the sarcoplasmic reticulum and increase the rate of Ca^{2+} uptake into the sarcoplasmic reticulum, or several hydrophobic fragments of the same, which increase the systolic Ca^{2+} release from the sarcoplasmic

reticulum and increase the rate of Ca^{2+} uptake into the sarcoplasmic reticulum.

21. A use of one or more nucleic acid molecules comprising sequences which code for one or more S100 proteins for the preparation of a medicament for the treatment of cardiac insufficiency, wherein the one or more S100 proteins are selected from the group consisting of S100A1 as shown in SEQ ID NO:2, S100A2 as shown in SEQ ID NO:4, S100A3 as shown in SEQ ID NO:6, S100A4 as shown in SEQ ID NO:8, S100A5 as shown in SEQ ID NO:10, S100A6 as shown in SEQ ID NO:12, S100A7 as shown in SEQ ID NO:14, S100A8 as shown in SEQ ID NO:16, S100A9 as shown in SEQ ID NO:18, S100A10 as shown in SEQ ID NO:20, S100A11 as shown in SEQ ID NO:22, S100A12 as shown in SEQ ID NO:24, S100A13 as shown in SEQ ID NO:26 and S100β as shown in SEQ ID NO:28, one or more mutants of the same having an identity of at least 60% thereto which increase the systolic Ca^{2+} release from the sarcoplasmic reticulum and increase the rate of Ca^{2+} uptake into the sarcoplasmic reticulum, or several hydrophobic fragments of the same, which increase the systolic Ca^{2+} release from the sarcoplasmic reticulum and increase the rate of Ca^{2+} uptake into the sarcoplasmic reticulum.

22. A use of a gene transfer vector which contains nucleic acid sequences which code for one or more S100 proteins for the preparation of a medicament for the treatment of cardiac insufficiency, wherein the one or more S100 proteins are selected from the group consisting of S100A1 as shown in SEQ ID NO:2, S100A2 as shown in SEQ ID NO:4, S100A3 as shown in SEQ ID NO:6, S100A4 as shown in SEQ ID NO:8, S100A5 as shown in SEQ ID NO:10, S100A6 as shown in SEQ ID NO:12, S100A7 as shown in SEQ ID NO:14, S100A8 as shown in SEQ ID NO:16, S100A9 as shown in SEQ ID NO:18, S100A10 as shown in SEQ ID NO:20, S100A11 as shown in SEQ ID NO:22, S100A12 as shown in SEQ ID NO:24, S100A13 as shown in SEQ ID NO:26 and S100β as shown in SEQ ID NO:28, one or more mutants of the same having an identity of at least 60% thereto which increase

the systolic Ca^{2+} release from the sarcoplasmic reticulum and increase the rate of Ca^{2+} uptake into the sarcoplasmic reticulum, or several hydrophobic fragments of the same, which increase the systolic Ca^{2+} release from the sarcoplasmic reticulum and increase the rate of Ca^{2+} uptake into the sarcoplasmic reticulum.

23. The use according to claim 22, wherein the S100 protein comprises S100A1 comprising the amino acid sequence displayed in SEQ ID NO: 2.

24. The use according to claim 22, wherein the fragments are selected from the amino acid sequence displayed in SEQ ID NO: 32, 34 or 36.

25. The use according to any one of claims 15 to 22, wherein a combination of at least two of the fragments represented in SEQ ID NO: 32, 34, 36, 37, 38 and 39 is used.

26. The use according to any one of claims 16 to 22, wherein the nucleic acid sequence coding for S100A1 according to SEQ ID NO: 1 or a gene transfer vector containing this sequence is used.

27. The use according to any one of claims 16 to 22, wherein the nucleic acid sequences coding for the fragments are selected from SEQ ID NO: 31, 33 or 35 or gene transfer vectors containing these sequences.

28. The use according to any one of claims 16 to 22, wherein a combination of at least two of the nucleic acid sequences coding for SEQ ID NO: 32, 34, 36, 37, 38 and 39, or at least two of the nucleic acid sequences represented in SEQ ID NO: 31, 33, 35 and sequences coding for SEQ ID NO: 37, 38 and 39, or one or more gene transfer vectors containing this combination is used.

29. The use according to any one of claims 15 to 28, wherein the medicament is formulated for oral, intravenous, intraarterial or intracoronal application.

30. The use according to any one of claims 15 to 29, wherein the medicament

is formulated with pharmaceutically compatible auxiliaries or supports.

31. An isolated peptide consisting of the sequence represented in SEQ ID NO: 32, 34, 36, 37, 38 or 39.

Fig. 1a

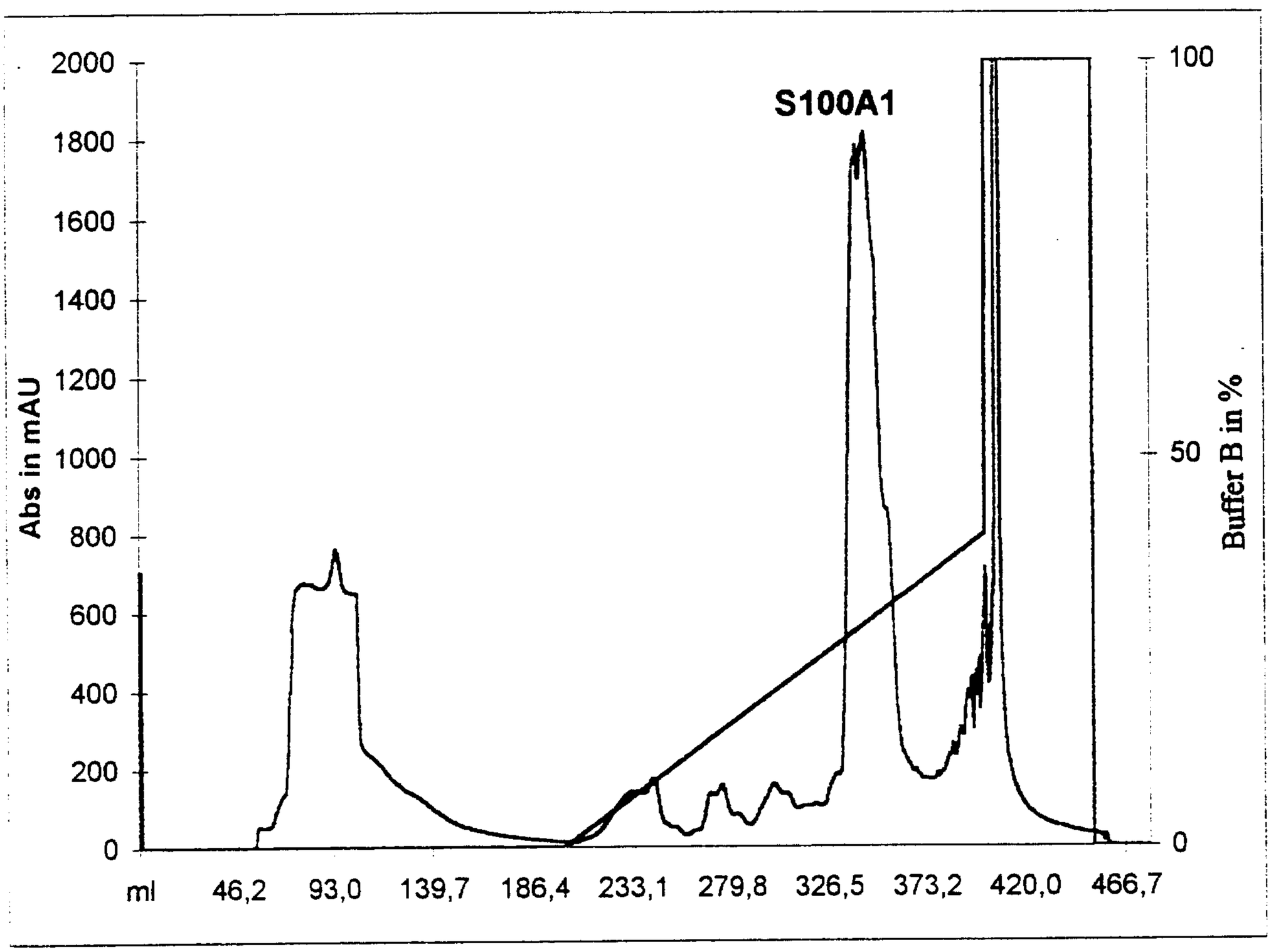
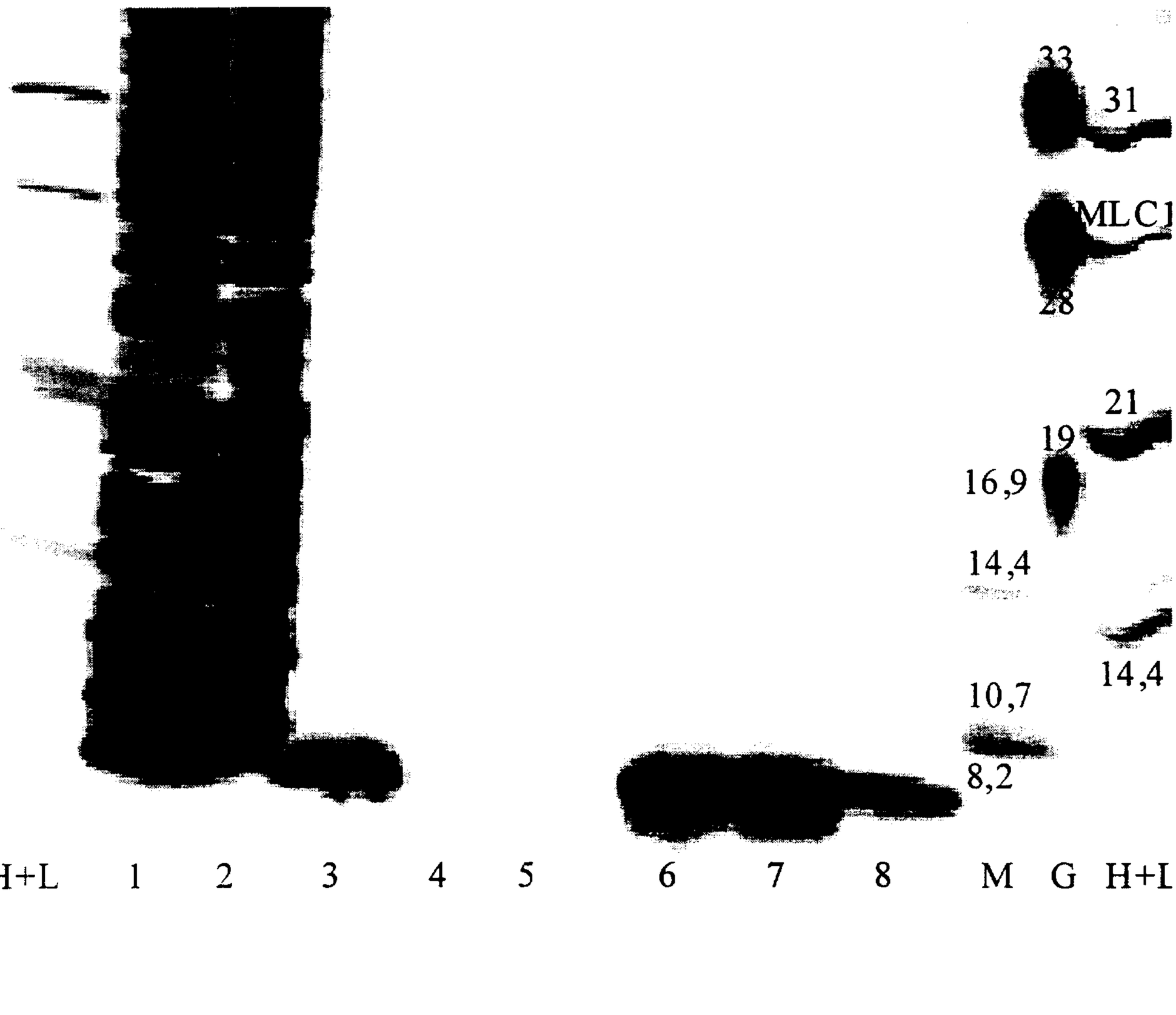

S100A1
Abs in mAU
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Buffer B in %
100
50
0
ml
46,2
93,0
139,7
186,4
233,1
279,8
326,5
373,2
420,0
466,7

Fig. 1b



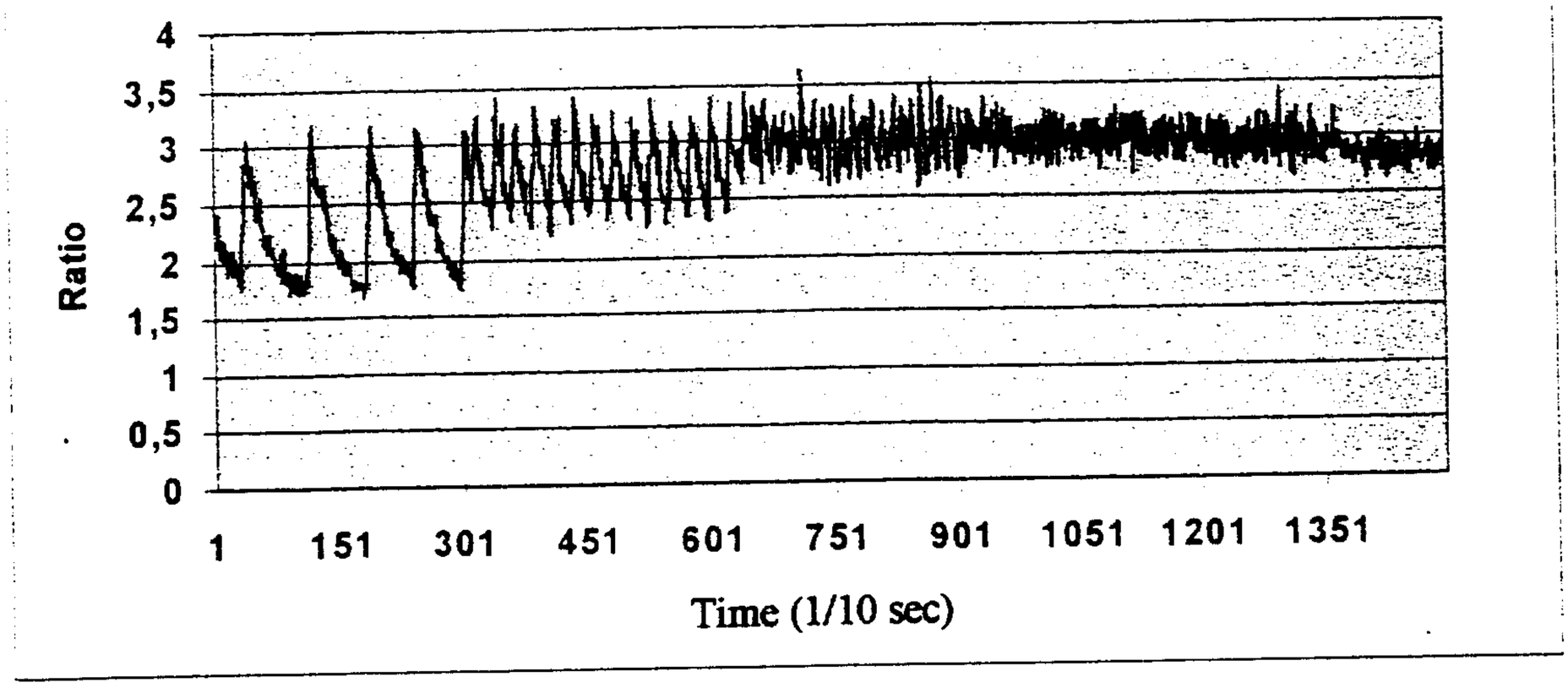

Neonatal cardiomyocytes control
Natural frequency /30bpm/60bpm/120bpm/300bpm
Ratio
4
3,5
3
2,5
2
1,5
1
0,5
0
1
151
301
451
601
751
901
1051
1201
1351
Time (1/10 sec)

Fig. 2a

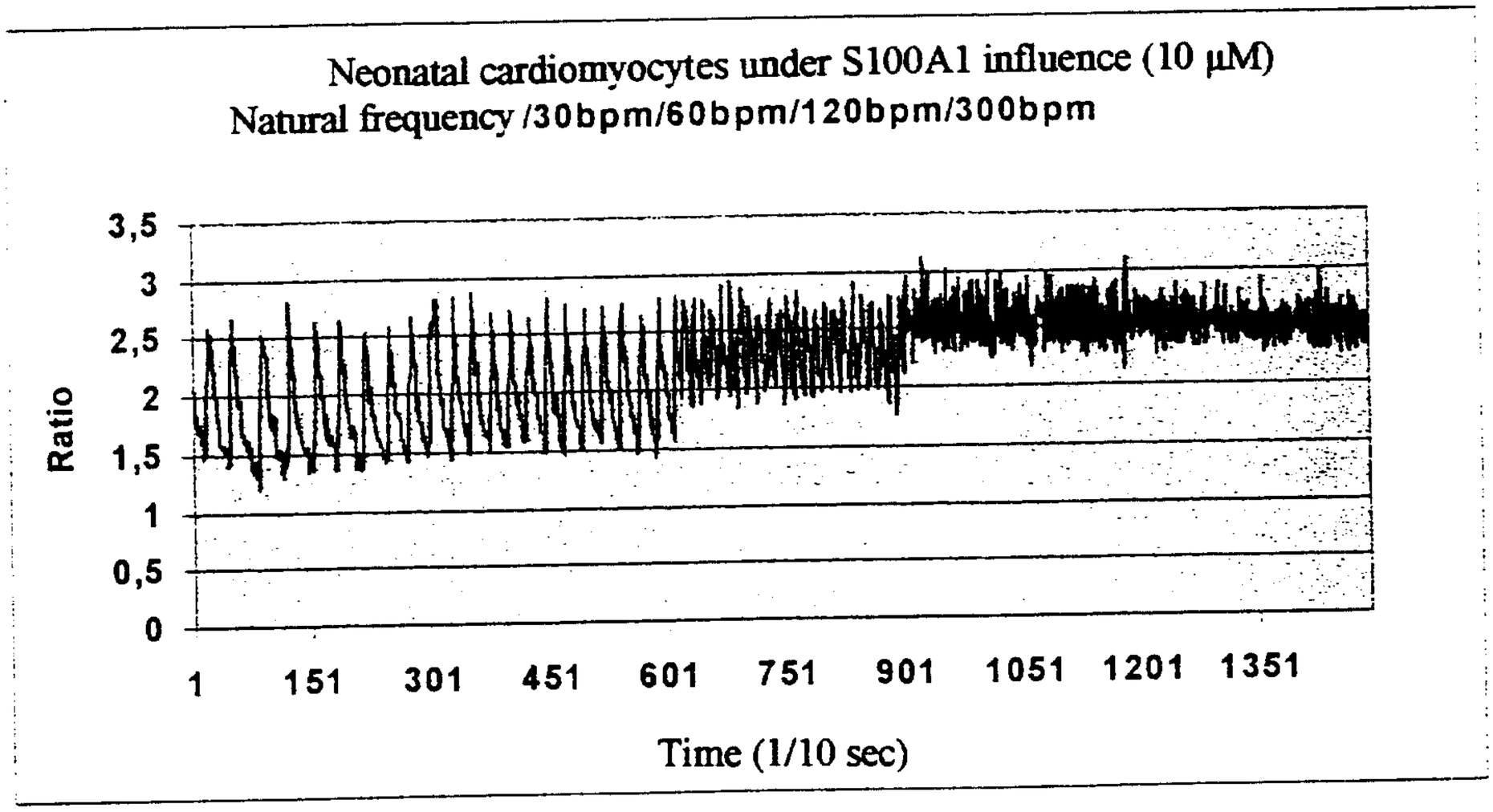

Neonatal cardiomyocytes under S100A1 influence (10 µM)
Natural frequency /30bpm/60bpm/120bpm/300bpm
Ratio
3,5
3
2,5
2
1,5
1
0,5
0
1
151
301
451
601
751
901
1051
1201
1351
Time (1/10 sec)

Fig. 2b

Fig. 3

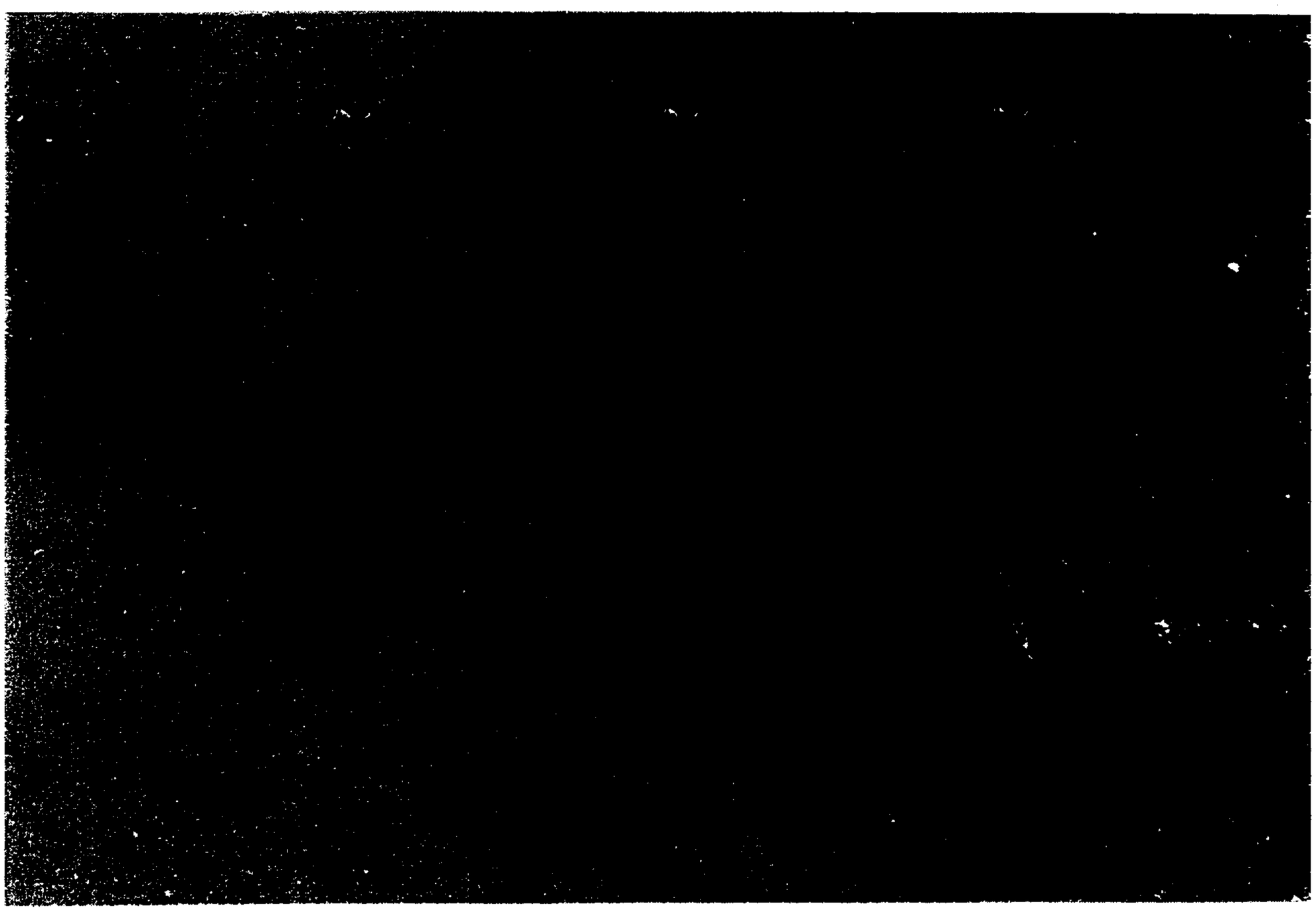


Fig. 4a

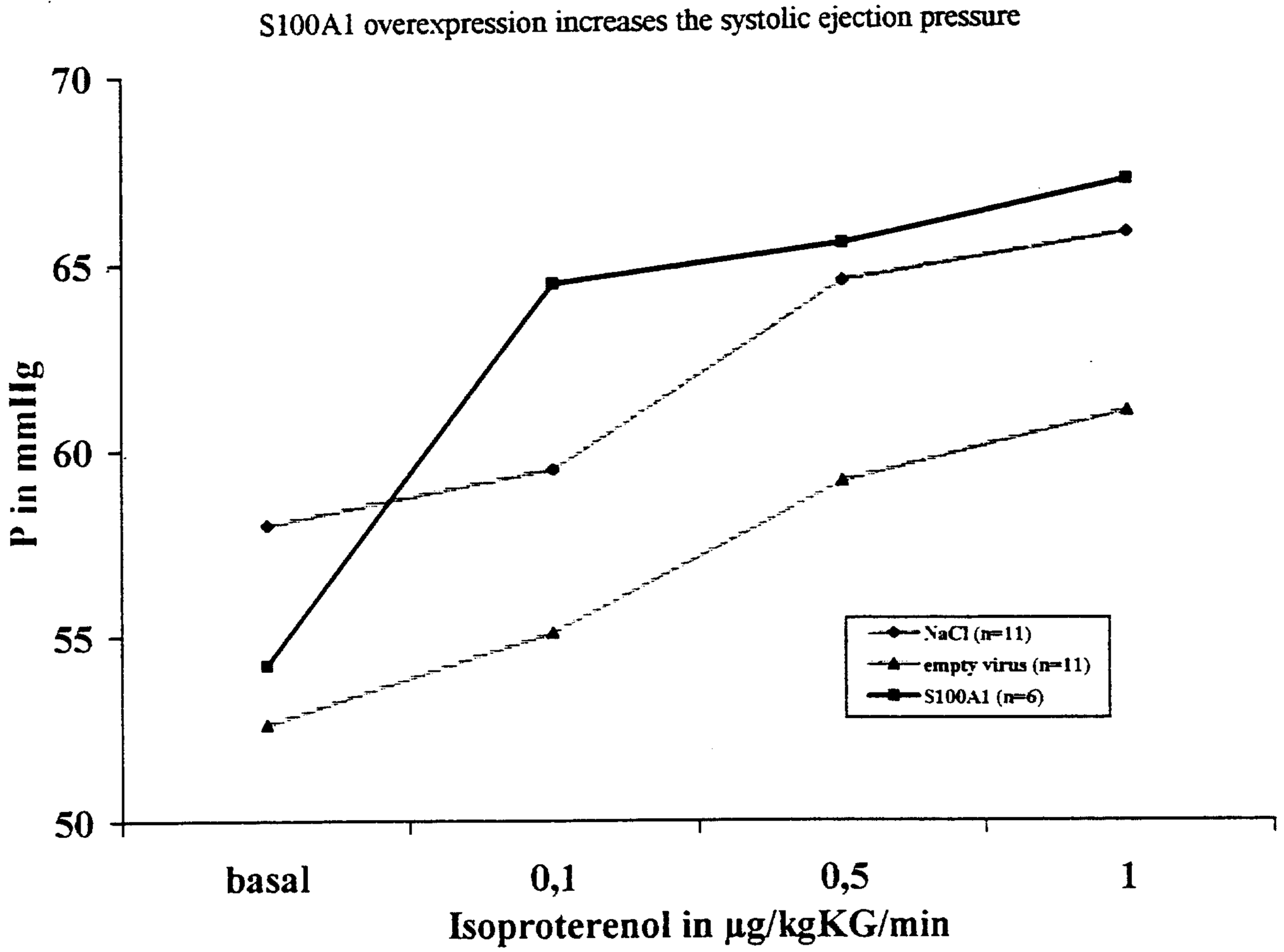

S100A1 overexpression increases the systolic ejection pressure
70
65
60
55
50
P in mmHg
basal
0,1
0,5
1
Isoproterenol in µg/kgKG/min
NaCl (n=11)
empty virus (n=11)
S100A1 (n=6)

Fig. 4 b

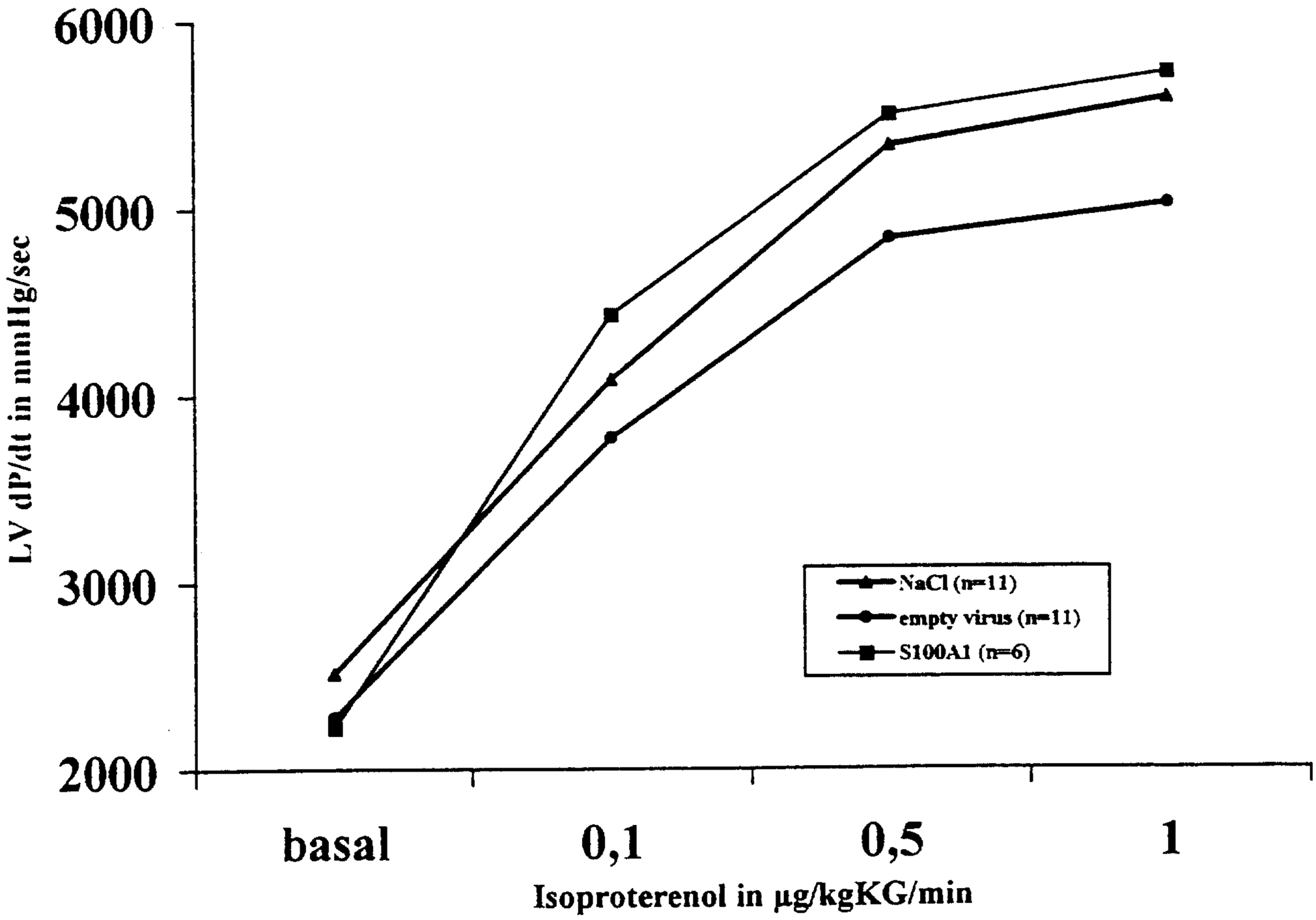

S100A1 increases the velocity of contraction
6000
5000
4000
3000
2000
LV dP/dt in mmHg/sec
NaCl (n=11)
empty virus (n=11)
S100A1 (n=6)
basal
0,1
0,5
1
Isoproterenol in µg/kgKG/min

Fig. 5a

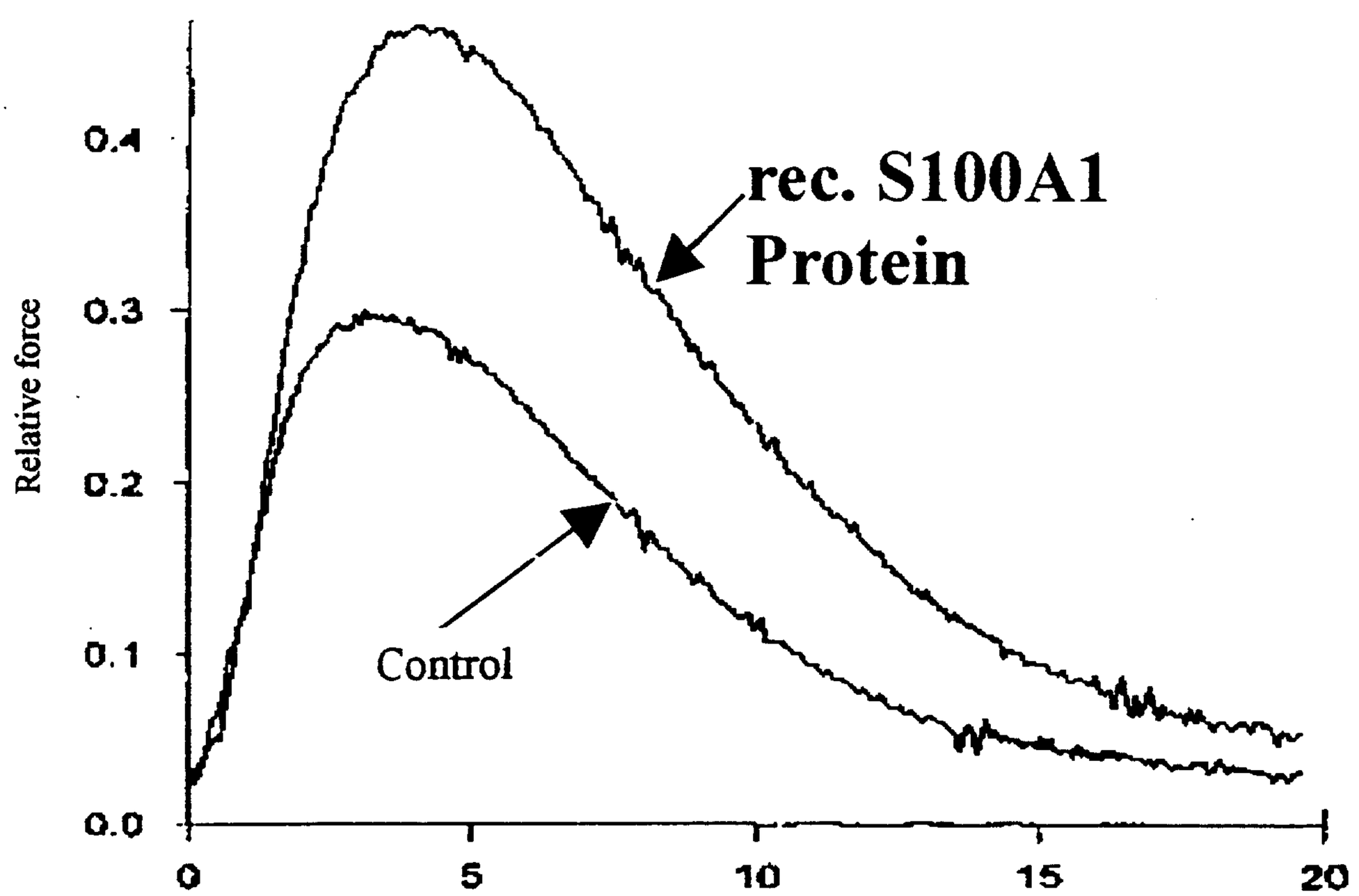
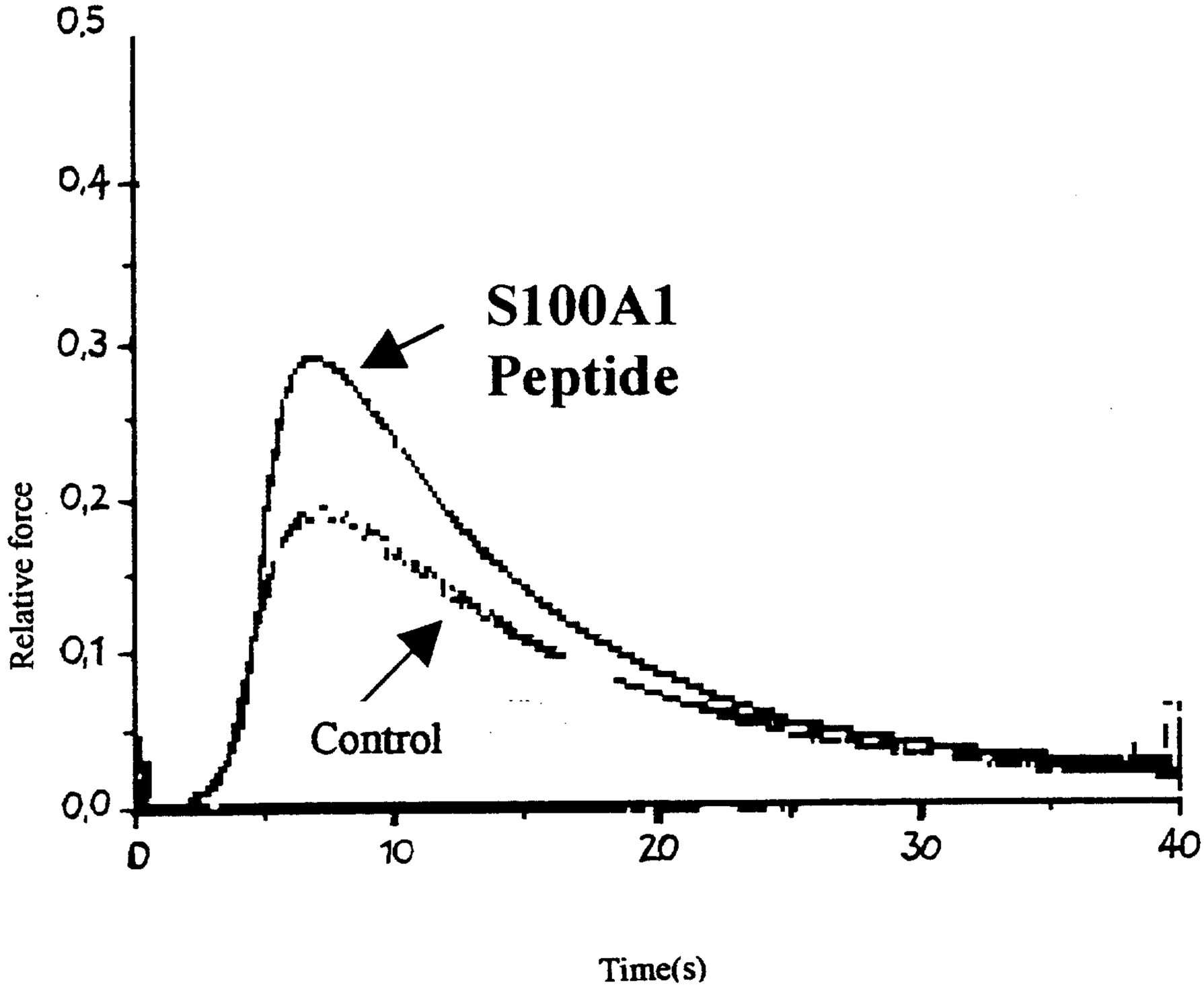

0.5
0.4
0.3
0.2
0.1
0.0
Relative force
rec. S100A1
Protein
Control
0
5
10
15
20
Time(s)

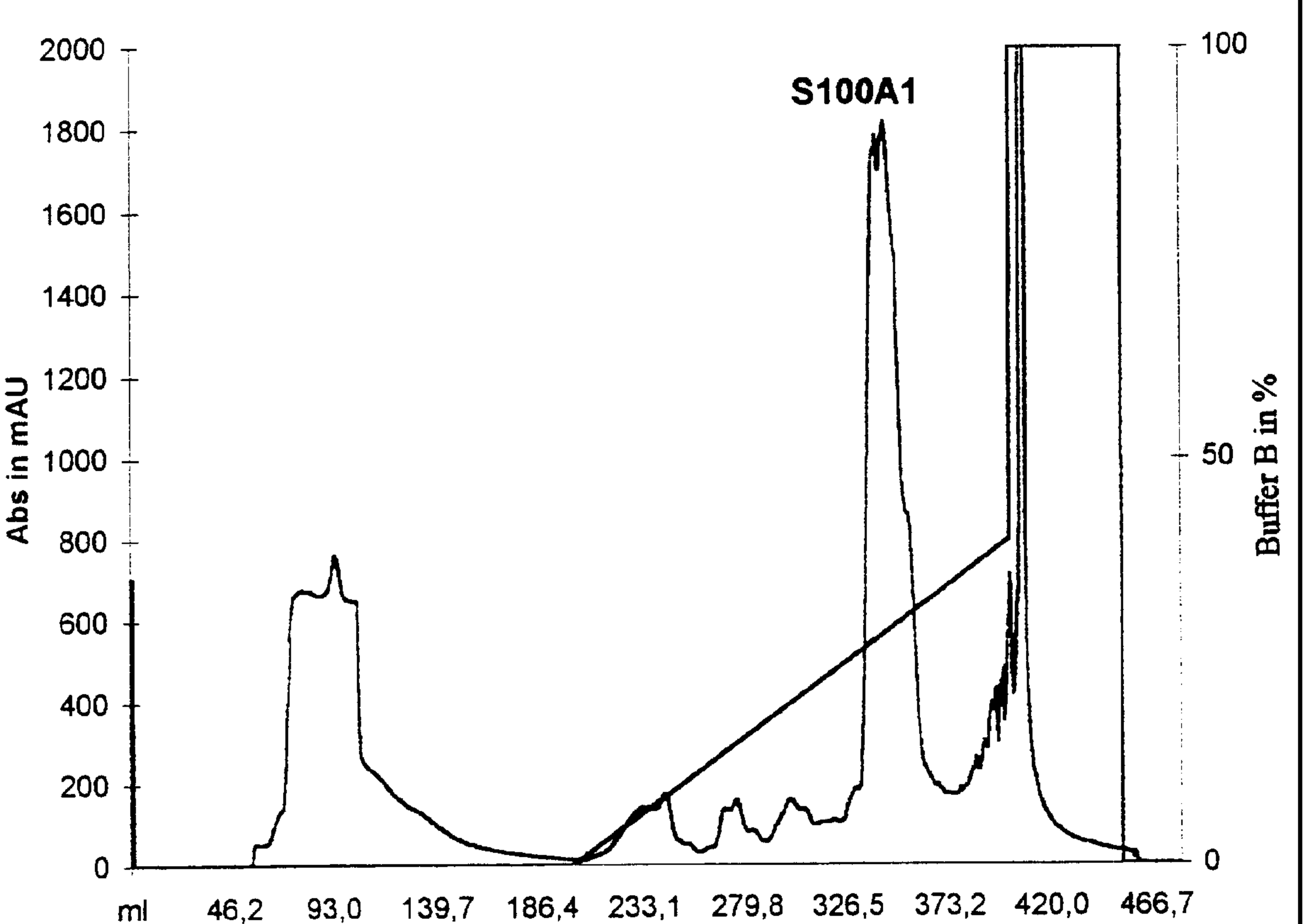
Fig. 5 b


0,5
0,4
0,3
0,2
0,1
0,0
Relative force
S100A1
Peptide
Control
0
10
20
30
40
Time(s)

Application number/ Numéro de demande : EP00/02453

Documents of poor quality scanned
(request original documents in File Prep. Section on the 10th floor)

Documents de piètre qualité numérisés
(Pour obtenir les documents originaux, veuillez vous adresser à la Section de préparation des dossiers, située au 10e étage)


S100A1
Abs in mAU
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Buffer B in %
100
50
0
ml
46,2
93,0
139,7
186,4
233,1
279,8
326,5
373,2
420,0
466,7