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(54) Title: PARATHYROID HORMONE ANALOGUES AND USE IN OSTEOPOROSIS TREATMENT

(57) Abstract

Analogues of bovine and human parathyroid hormone, wherein the twenty-third amino acid of the natural hormone, tryptophane, has been substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Lys, Met, Pro, Ser or Thr have been found to retain bone cell effect with minimal effects on blood pressure and smooth muscle, including cardiac muscle. It has further been found that this effect can be obtained by using a synthetic PTH containing only the first 34 amino acids of PTH, with substitution at the twenty-third amino acid as described. These analogues of PTH also are effective in the treatment of osteoporosis and other bone diseases.

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PARATHYROID HORMONE ANALOGUES AND USE IN OSTEOPOROSIS TREATMENT

FIELD OF THE INVENTION

This invention relates to analogues of parathyroid hormone which, by substitution at the twenty-third position of natural parathyroid hormone, have been found to affect calcium change in bone cells without producing the typical effects of parathyroid hormone on systolic and diastolic blood pressure, the effects on smooth muscle relaxation, vascular smooth muscle calcium change as well as positive chronotropic and inotropic effects on the heart.

BACKGROUND OF THE INVENTION

Parathyroid hormone (hereinafter, PTH) is produced by the parathyroid gland and is involved in the control of calcium levels in blood. It is a hypercalcemic hormone, elevating blood calcium levels. PTH is a polypeptide and the amino acid sequences of bovine and human PTH are closely related. Only the residues at locations one, seven and sixteen differ between the two. Synthetic polypeptides containing the first thirty-four residues of PTH may be prepared using the method disclosed by Erickson and Merrifield, The Proteins, Neurath et al., Eds., Academic Press, New York, 1976, page 257, preferably as modified by the method of Hodges et al., Peptide Research, 1, 19 (1988).

When serum calcium is reduced to below a "normal" level, the parathyroid gland releases PTH and resorption of bone calcium and increased absorption of calcium from the intestine, as well as renal reabsorption of calcium, occur.

The antagonist of PTH is calcitonin, which acts to reduce the level of circulating calcium. PTH is known to stimulate osteoclasts and its activity requires the presence of derivatives of vitamin D_3, especially 1,25-

dihydroxycholecalciferol.

Intracellular calcium, particularly in the cells of the vascular system, has been shown to affect changes in vascular tension, as can be measured by changes in blood pressure. U.S. Patent Application 603,745 describes one method which has been discovered to regulate calcium uptake in vascular cells.

Osteoporosis is a progressive disease which is particularly characteristic of postmenopausal women, and results in the reduction of total bone mass. The sequelae frequently involve fractures of load-bearing bones and the physical degenerations characteristic of immobilizing injuries. Osteoporosis is associated with hyperthyroidism, hyperparathyroidism, Cushings syndrome and the use of certain steroidal drugs. Remedies historically have involved increase in dietary calcium, estrogen therapy and increased doses of vitamin D.

PTH has been used to treat osteoporosis. However, while the use of PTH is effective in the treatment of osteoporosis by diminishing the loss of bone mass, PTH may exhibit other undesired pharmalogical effects, such as hypotension and smooth muscle relaxation (e.g. relaxation of gastrointestinal organs, uterus, tracheal and vas deferens) as well as positive chronotropic and inotropic effects on the heart. The relaxation effects of PTH on smooth muscle as well as positive chronotropic and inotropic effects of PTH are described in Pang et al, Trends in Pharmacological Sciences, Vol. 7, No. 9, pp. 340-341 (September 1986).

U.S. Patent No. 4,771,124 discloses the property of bovine and human PTH analogues wherein Trp^{23} is substituted by amino acids phenylalanine, leucine, norleucine, valine, tyrosine, beta-naphtylalanine and alpha-naphtylalanine as a PTH antagonist. While it was suggested that these analogues might be useful in the treatment of osteoporosis, it was based on the analogues antagonistic action to PTH. Furthermore, there was no data to indicate the effectiveness these analogues on bone or other tissue. In addition, analogues with substituted at

Trp^{23} with leucine, phenylalanine or tyrosine would produce undesired secondary effects of smooth muscle relaxation, vascular smooth muscle calcium change as well as positive chronotropic and inotropic effects on the heart.

Because PTH is a peptide, topical administration would be the preferred method of administration. However, topical application of PTH or the aforementioned analogues which exhibit vasoactivity would likely produce an undesired local vascular reaction. This reaction could be potentially detrimental if, for example, nasal administration is employed.

It is one object of this invention to ameliorate bone loss while preventing smooth muscle relaxation as well as positive chronotropic and inotropic effects on the heart and without significantly changing blood pressure. It is another object of this invention to identify that portion of PTH which is responsible for calcium regulation and that portion which appears to be primarily related to control of blood pressure and smooth muscle action.

BRIEF SUMMARY OF THE INVENTION

Modification of either bovine or human PTH at the twenty-third amino acid position to substitute for tryptophane either alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, lysine, methionine, proline, serine or threonine produces essentially no change in systolic and diastolic blood pressure, no change in smooth muscle tension and no change in the rate and force of contraction of the heart as compared to native PTH. It also has been observed that the PTH analogue containing only the first thirty-four amino acids, with substitution at the twenty-third position, is equally effective in increasing the "osteo effect" without changing blood pressure or causing smooth muscle relaxation or positive chronotropic and inotropic effects on the heart.

The analogues of the present invention should be effective in ameliorating bone loss while preventing smooth muscle relaxation as well as positive chronotropic and inotropic effects on the heart and without significantly changing blood pressure.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1a shows the structure of natural bovine PTH (SEQ ID NO:1).

Fig. 1b shows the structure of natural human PTH (SEQ ID NO:2).

Fig. 2a shows the structure of bPTH (1-34) with position 23 substituted with Xaa (SEQ ID NO:3).

Figs. 2b-2p show the structure of bPTH (1-34) with position 23 substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Lys, Met, Pro, Ser or Thr, respectively (SEQ ID NO:4 - SEQ ID NO:18).

Fig. 3a shows the structure of hPTH (1-34) with position 23 substituted with Xaa (SEQ ID NO:19).

Figs. 3b-3p show the structure of hPTH (1-34) with position 23 substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Lys, Met, Pro, Ser or Thr, respectively (SEQ ID NO:20 - SEQ ID NO:34).

Fig. 4 shows the structure of bPTH with position 23 substituted with Xaa (SEQ ID NO:35).

Fig. 5 shows the structure of hPTH with position 23 substituted with Xaa (SEQ ID NO:36).

Fig. 6 shows the effect of bPTH-(1-34) and its analogues on diastolic blood pressure of anesthetized Sprague-Dawley rats. Cs114 had no effect.

Fig. 7 shows the effect of bPTH-(1-34) and its analogues on systolic blood pressure of anesthetized Sprague-Dawley rats. Cs114 had no effect.

Fig. 8 shows the vasorelaxing effect of bPTH-(1-34) and its analogues on rat tail artery helical strip in vitro. Cs114 had no effect.

Fig. 9 shows the depolarizing concentrations of KCl which increased calcium ion levels in cultured osteoblasts. Drug 788 is an anti-osteoporotic agent which inhibits the KCl effect.

Figs. 10 a-d show the depolarizing concentrations of KCl which increased calcium levels in cultured osteoblasts. Addition of bPTH-(1-34) inhibits the KCl effect.

Figs. 11 a-c show the depolarizing concentrations of KCl increasing calcium ion levels in cultured osteoblasts. Cs114 inhibits the KCl effect.

Fig. 12 shows the relation between the relaxation curves of Sprague-Dawley rat tail artery helical strips, precontracted with AVP when treated with Cs114, Cs117 and Cs201.

Fig. 13 shows the effects illustrated in Fig. 12, using Cs206, Cs207, Cs501, Cs502 and Cs503.

Fig. 14 shows the relaxation curves produced by Cs117, Cs201, Cs501, Cs502 and Cs503 on rat tail artery helical strips precontracted with KCl.

Fig. 15 shows the relationship between the tension of rat tail artery helical strips depolarized with 10^{-7} M NE, as a function of calcium concentration in the presence of Cs114.

Fig. 16 shows the effect of Cs114 on the intracellular calcium concentration in the presence of KCl in UMR cells in culture.

Fig. 17 shows a comparison of the effect of Cs205 and bPTH on the mean arterial blood pressure of anesthetized Sprague-Dawley rats.

Fig. 18 shows the dose-response relationship between Cs205 and the tension of rat tail artery helical strips precontracted with KCl, norepinephrine and AVP.

Fig. 19 shows a comparison of the effect of Cs201 and bPTH on the mean arterial blood pressure of anesthetized Sprague-Dawley rats.

Fig. 20 shows the dose-response relationship between Cs201 and the tension of rat tail artery helical strips precontracted with KCl, norepinephrine and AVP.

Fig. 21 shows a comparison of the effect of Cs503 and bPTH on the mean arterial blood pressure of anesthetized Sprague-Dawley rats.

Fig. 22 shows the dose-response relationship between Cs503 and the tension of rat tail artery helical strips precontracted with KCl, norepinephrine and AVP.

Fig. 23 shows a comparison of the effect of Cs502 and bPTH on the mean arterial blood pressure of anesthetized Sprague-Dawley rats.

Fig. 24 shows the dose-response relationship between Cs502 and the tension of rat tail artery helical strips precontracted with KCl, norepinephrine and AVP.

Fig. 25 shows a comparison of the effect of Cs501 and bPTH on the mean arterial blood pressure of anesthetized Sprague-Dawley rats.

Fig. 26 shows the dose-response relationship between Cs501 and the tension of rat tail artery helical strips precontracted with KCl, norepinephrine and AVP.

Fig. 27 comparison of the effect of Cs207 and bPTH on the mean arterial blood pressure of anesthetized Sprague-Dawley rats.

Fig. 28 shows the dose-response relationship between Cs207 and the tension of rat tail artery helical strips precontracted with KCl, norepinephrine and AVP.

Fig. 29 shows the effect of Cs207 on intracellular calcium increase stimulated by KCl in cultured UMR osteoblast cells.

Fig. 30 shows the effect of Cs207 on intracellular calcium increase stimulated by KCl in cultured UMR cells.

Fig. 31 shows a comparison of the effect of Cs206 and bPTH on the mean arterial blood pressure of anesthetized Sprague-Dawley rats.

Fig. 32 shows the dose-response relationship between Cs206 and the tension of rat tail artery helical strips precontracted with KCl, norepinephrine and AVP.

Fig. 33 shows the effect of Cs206 on intracellular calcium concentration stimulated by KCl in cultured UMR osteoblast cells.

Fig. 34 shows the effect of Cs206 on intracellular calcium concentration stimulated by KCl in cultured UMR cells.

Fig. 35 shows a comparison of the effect of Cs114 and bPTH on the systolic blood pressure of anesthetized Sprague-Dawley rats.

Fig. 36 shows a comparison of the effect of Cs114 and bPTH on the diastolic blood pressure of anesthetized Sprague-Dawley rats.

Fig. 37 shows the dose-response relationship between Cs114 and the tension of rat tail artery helical strips precontracted with KCl, norepinephrine and AVP.

Fig. 38 shows the effect of Cs114 on intracellular calcium concentration stimulated by KCl in cultured UMR osteoblast cells.

Fig. 39 shows the effect of Cs114 (A) on intracellular calcium concentration stimulated by KCl in cultured UMR cells at 15 mM KCl.

Fig. 40 shows the effect of Cs114 (B) on intracellular calcium concentration stimulated by KCl in cultured UMR cells at 30 mM KCl.

Fig. 41 shows the effect of Cs88 [bPTH-(1-34)] on the mean arterial blood pressure of anesthetized Sprague-Dawley rats.

Fig. 42 shows the dose-response relationship between Cs88 [bPTH-(1-34)] and the tension of rat tail artery helical strips precontracted with KCl, norepinephrine and AVP.

Fig. 43 shows the effect of Cs88 on intracellular calcium concentration stimulated by KCl in cultured UMR osteoblast cells.

Fig. 44 shows the effect of Cs88 on intracellular calcium concentration stimulated by KCl in cultured UMR cells.

Fig. 45 shows the effect of Cs113 on the intracellular calcium concentration stimulated by KCl in the presence of KCl in UMR cells in culture.

Fig. 46 shows the effect of Cs501 on the intracellular calcium concentration stimulated by KCl in the presence of KCl in UMR cells in culture.

Fig. 47 shows the effect of Cs1001 on the intracellular calcium concentration stimulated by KCl in the presence of KCl in UMR cells in culture.

Fig. 48 shows the effect of Cs114 on the contractility and contraction rate of right atrial tissue of Sprague-Dawley rats.

Fig. 49 shows the effect of Cs201 on the contractility and contraction rate of right atrial tissue of Sprague-Dawley rats.

Fig. 50 shows the effect of Cs205 on the contractility and contraction rate of right atrial tissue of Sprague-Dawley rats.

Fig. 51 shows the effect of Cs206 on the contractility and contraction rate of right atrial tissue of Sprague-Dawley rats.

Fig. 52 shows the effect of Cs207 on the contractility and contraction rate of right atrial tissue of Sprague-Dawley rats.

Fig. 53 shows the effect of Cs208 on the contractility and contraction rate of right atrial tissue of Sprague-Dawley rats.

Fig. 54 shows the effect of Cs209 on the contractility and contraction rate of right atrial tissue of Sprague-Dawley rats.

Fig. 55 shows the effect of Cs211 on the contractility and contraction rate of right atrial tissue of Sprague-Dawley rats.

Fig. 56 shows the effect of Cs212 on the contractility and contraction rate of right atrial tissue of Sprague-Dawley rats.

Fig. 57 shows the effect of Cs213 on the contractility and contraction rate of right atrial tissue of Sprague-Dawley rats.

Fig. 58 shows the effect of Cs214 on the contractility and contraction rate of right atrial tissue of Sprague-Dawley rats.

Fig. 59 shows the effect of Cs215 on the contractility and contraction rate of right atrial tissue of Sprague-Dawley rats.

Fig. 60 shows the effect of Cs220 on the contractility and contraction rate of right atrial tissue of Sprague-Dawley rats.

Fig. 61 shows the effect of Cs501 on the contractility and contraction rate of right atrial tissue of Sprague-Dawley rats.

Fig. 62 shows the effect of Cs503 on the contractility and contraction rate of right atrial tissue of Sprague-Dawley rats.

Fig. 63 shows the effect of Cs2001 and Cs1001 on the contractility and contraction rate of right atrial tissue of Sprague-Dawley rats.

Fig. 64 shows the effect of Cs219 on the contractility and contraction rate of right atrial tissue of Sprague-Dawley rats.

Fig. 65 shows the effect of Cs218 on the contractility and contraction rate of right atrial tissue of Sprague-Dawley rats.

Fig. 66 shows the effect of Cs502 on the contractility and contraction rate of right atrial tissue of Sprague-Dawley rats.

DETAILED DESCRIPTION OF THE INVENTION

There are at least two known catagories of functions for PTH. PTH is involved in calcium balance in the blood stream and controls both the amount of calcium uptake from the gastrointestinal tract and the deposition and removal of calcium from bone. Calcium also has been found to be effective in the maintenance of blood pressure. Cox, J. Cardiovascular Pharmacology, Vol. 8 (1986), Supp. 8 S48. Control of calcium in the walls of blood vessels is a useful therapeutic regimen for controlling hypertension and calcium channel blockers, which prevent the introduction of calcium into cell walls, is a conventional therapy for hypertension. Needleman et al. in Goodman and Gilman's The Pharmacological Basis of Therapeutics, MacMillan, New York, (1985), page 816 ff.

Administration of therapeutic doses of PTH has been found to be effective for the control of osteoporosis, particularly in individuals who have been subjected to thyroidectomies/ parathyroidectomies. Therapeutic dosages of PTH will, in some individuals, result in unacceptable diminution of blood pressure and may result in relaxation of smooth muscles such as gastrointestinal, uterus, tracheal, vas deferens as well as exhibit positive chronotropic and inotropic effects on the heart. To avoid hypotensive effects, smooth muscle relaxation effects and positive chronotropic and inotropic effects on the heart, it was envisaged that the structure of PTH could be modified to decouple the hypotensive, smooth muscle relaxation and positive chronotropic and inotropic function from the bond calcium and bone deposition function. It has now been discovered that a critical site exists at amino acid twenty-three, which is tryptophane (Trp) in both bovine and human PTH. Substitution at the Trp site with other amino acids diminishes the hypotensive, smooth muscle relaxation and positive chronotropic and inotropic effects without denigrating from the osteo effect. Particularly effective in this regard, is the substitution of alanine (Ala) or other amino acids for Trp.

The procedure of Erickson and Merrifield, as modified by

Hodges et al., as described above, may be used to synthesize synthetic PTH or fragments thereof. The procedure enables substitution for the naturally occurring PTH at substantially every location and it is possible to prepare both bovine and human synthetic PTH at full length or in the sequence of the first thirty-four amino acids, which is more facilely performed. Such substitution can also be accomplished by genetic engineering.

Substitution at position twenty-three invariably alters the observed hypotensive, smooth muscle relaxation and positive chronotropic and inotropic effects, whether the full length PTH or the 1-34 fragment is administered. Substitution of Ala for Trp at position twenty-three is particularly preferred because the change in blood pressure, smooth muscle relaxation and positive chronotropic and inotropic effects from this substitution are minimal and calcium uptake, as measured in osteoblasts, mimics the results from the administration of native PTH. The 1-34 PTH fragment with Ala^{23} or other amino acids is particularly preferred because the pharmacological properties are those which are desired and the difficulty of synthesis is minimized. Synthesis of the compounds used in the development of this invention was performed at Alberta Peptide Institute (API) and the cooperation of API is gratefully acknowledged.

The structure of bovine parathyroid hormone (bPTH) and human parathyroid hormone (hPTH) are shown in Figs. 1a (SEQ ID NO:1) and 1b (SEQ ID NO:2). Representative synthetic analogues are described in Table 1 and are further shown in Figs. 2-5 and SEQ ID NO:3-SEQ ID NO:36. The hypotensive effects of these analogues is shown in Figs. 6, 7, 17, 19, 21, 23, 25, 27, 31, 35, 36, and 41. All of the analogues produce either no or less diminution of blood pressure than does native PTH. The Ala^{23} analogue provides almost no change in blood pressure, either systolic or diastolic, over a range of 0-5 $\mu g/kg$. At the level of 5 $\mu g/kg$ of PTH, the blood pressure in Sprague-Dawley rats is such that they are essentially moribund.

We have developed a method for modeling the hypotensive effects of natural and synthetic chemical compounds using helically cut tail arteries from Sprague-Dawley rats in a Sawyer-Bartlestone chamber, measuring the change in tension with a force displacement transducer. This method and the effect of bovine PTH-(1-34) in this system is described in Blood Vessels, 22, 57 (1985). It is demonstrated in this paper that bPTH-(1-34) produces dose-dependent relaxation of helical strips of rat tail artery which have been previously contracted using arginine-vasopressin (AVP). Figs. 8, 12, 15, 18, 20, 22, 24, 26, 28, 32, 37 and 42 illustrate the effect of the PTH analogues of this invention as measured using this in vitro technique. Alternatively, the strips may be precontracted using other pressor substances such as norepinephrine (NE) or KCl.

We have also developed a method of modeling the chronotropic effects of natural and synthetic chemicals using the right atrium from Sprague-Dawley rats and measuring the change in the force and rate of atrium contraction. This method and the effects of bovine PTH (1-34) in this system are described in Tenner et al, The Canadian Journal of Physiology and Pharmacology, Volume 61, No. 10 (1983) pp. 1162-1167. It is demonstrated in this paper that bPTH (1-34) produces significant dose-dependent chronotropic effects on rat cardiac pacemaker tissue. Figs. 48-66 illustrate the effect of the PTH analogues of this invention as measured using this in vitro technique.

Because osteoporosis is a progressive syndrome, a model is required and the use of cultured osteoblasts of the UMR-106 rat osteosarcoma cells, ATCC CRL 1661 have been used as the model. Intracellular calcium concentration change in these cells has been monitored using the FURA-2 method, wherein a fluorescent dye which is specific for calcium is used as a marker for calcium uptake into the cells. Cells are incubated with 1-10 μM of the acetomethoxy ester of FURA-2 for 30-60 minutes. Upon uptake, the ester is hydrolyzed to release free FURA-2, which selectively binds free Ca^{2+}. FURA-2 has a characteristic fluorescence spectrum, which wavelength is shifted when the dye binds to free Ca^{2+}. According to the method, Ca^{2+} which is present in the cell can be quantified by exciting the dye at two different wavelengths, 340 and 380 nm. The emission fluorescence is measured at 510 nm. The calcium concentration is proportional to the ratio of the fluorescent emission when excited at 340 nm to the emission at 380 nm. It is conventional to report the concentration of calcium within the cell in terms of the fluorescence ratio, the 340/380 ratio. This technique is described in Grynkiewicz et al., J. Biol. Chem., 260, 3440 (1985) and Pang et al., P. N. A. S. (USA), 87, 623 (1990).

Figs. 9, 10 a-d, 11 a-c, 16, 29, 30, 33, 34, 38, 39, 40, 43, 44 and 45-47 illustrate the results of the above-described measurements when inhibitors such as an anti-osteoporotic agent (788) or bPTH-(1-34) or Cs114 were used in the presence of KCl.

As can be readily seen from the figures, the PTH analogues, whether full length or 1-34, which contain anomalous amino acids at position twenty-three (most particularly those which contain Ala^{23}) do not effect a hypotensive and smooth muscle relaxation response, including positive chronotropic effects, but do inhibit calcium uptake as stimulated by KCl in osteoblasts, which indicates that these compounds would have the same effect on bone cells as PTH and would be useful in the treatment of osteoporosis in mammals and, particularly, in man, without the aformentioned deleterious side effects in the elderly.

While not being bound by any theory, it is suggested that substitution of Trp^{23} by other amino acids in 1-84 PTH and in the 1-34 analogues removes the vasodepressor, smooth muscle relaxation and positive chronotropic and inotropic effects of either bPTH or hPTH. The effect on KCl induced in osteoblasts, however, is essentially unchanged for 1-84 or 1-34 PTH. In other words, the effect on bone cells is unchanged from PTH.

The physiological significance of an inhibiting effect on the KCL induced calcium uptake in bone cells is not yet understood. One hypothesis is that the analogues interact fully with bone cell receptor activity. The fact that the same effect is seen for both PTH and the analogues disclosed herein suggests that the site of interaction with the osteoblast cell receptor is unchanged by the substitution.

The analogues of the present invention can be used in the treatment of osteoporosis and other bone related diseases and disorders involving bone cell calcium regulation.

The analogues of the present invention may be administered to a warm-blooded mammalian in need thereof, particularly a human, by parental, topical, rectal administration or by inhalation. The analogues may be conventionally formulated in a parenteral dosage form compounding about 1 to about 300 mg per unit of dosage with a conventional vehicle, excipient, binder, preservative, stabilizer, color, agent or the like as called for by accepted pharmaceutical practice.

For parental administration, a 1 to 10 ml intravenous, intramuscular or subcutaneous injection would be given one to four times daily. The injection would contain an analogue of the present invention in an aqueous isotonic sterile solution or suspension optionally with a preservative such as phenol or a solubilizing agent such as ethylenediaminetetraacetic acid (EDTA). Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. Synthetic monoglycerides, diglycerides, fatty acids (such as oleic acid) find use as fixed oil in the preparation of injectables.

For rectal administration, the analogues of the present invention can be prepared in the form of suppositories by mixing with a suitable non-irritating excipient such as cocoa butter or polyethylene glycols.

For topical use, the analogues of the present invention can be prepared in the form of ointments, jellies, solutions, suspensions or dermal adhesive patches.

In a powdered aerosol, analogues of the present invention may be administered by a spinhaler turbo-inhaler device obtained from Fisons Corporation of Bedford, Massachusetts, at a rate of about 0.1 to 50 mg per capsule, 1 to 8 capsules being administered daily for an average human. In a liquid aerosol, the compounds of the present invention are administered at the rate of about 100 to 1000 micrograms per "puff" or activated release of a standard volume of propellant. The liquid aerosol would be given at the rate of 1 to 8 "puffs" per day with variation in dosages due to the severity of the conditions being treated, the weight of the patient and the particle size distribution of the aerosol. A fluorinated hydrocarbon or isobutane find use as propellants for liquid aerosols.

Daily doses are in the range of about 0.01 to about 200 mg per kg of body weight, depending on the activity of the specific compound, the age, weight, sex and conditions of the subject to be treated, the type and severity of the disease, the frequency and route of administration. As would be well known, the amount of active ingredient that may be combined with the carried materials to produce a single dosage will vary depending upon the host treated and the particular mode of administration.

The following examples demonstrate the utility of applicants' invention. The examples are not limiting, but are illustrative only, and modifications which would be apparent to those skilled in the art are included within the scope of this disclosure.

Example 1

In Vivo Blood Pressure Measurement.

Sprague-Dawley (S-D) rats were anaesthetized with pentobarbital and a cannula was inserted into the carotid artery. The rats were kept sedated during the procedure and were injected with PTH peptides only when the blood pressure of the rats were stable. Peptides were injected through a cannula in the jugular vein, in amounts of 1, 3 and 5 or more μg/kg and the mean systolic and diastolic blood pressure was monitored continuously throughout the procedure. Results are reported with comparison to bPTH-(1-34).

Example 2

In Vitro Rat Tail Artery Helical Strip Tension Assay

The assay was performed according to Pang et al., Blood Vessels, 22, 57 (1985). Sprague-Dawley rats were anaesthetized with pentobarbital and the tail artery excised and placed in ice-cold Krebs-Hanseleit solution (KHS) oxygenated with 95% O_2, 5% CO_2. Each artery was cut helically and strips of approximately 1.5 cm were secured in a Sawyer-Bartlestone chamber containing KHS. The force generated by the strips was measured with a Grass FT03 force displacement transducer and recorded on a polygraph. Isolated tail artery helical strips were equilibrated for 1 hour prior to use.

One to two minutes prior to addition of a peptide, the strips were contracted by addition of either arginine vasopressin (AVP), potassium chloride (KCl) or norepinephrine (NE) to the bath. The peptide was then added to the bath and the degree of relaxation measured. Bovine serum albumin was used as a control. Results are reported as percent decrease in tension for each drug and dose used. Drug dose is calculated on the basis of the final concentration in the bath solution.

Example 3

In Vitro atrial contractility and contraction rate measurement

The assay was performed according to Tenner et al., Canadian Journal of Physiology and Pharmacology, Vol. 61, No. 10 (1983) pp. 1162-1167. Sprague-Dawley rats weighing between

100 and 250 g were treated with heparin (500 IU, i.p.) 15 minutes prior to decapitation. Thoracotomies were performed and the heart rapidly excised and placed in a cold physiological salt solution (PSS) having the following composition (in millimolar): NaCl, 120; KCl, 5.63; $CaCl_2$, 2.0; $MgCl_2$, 2.1; $NaHCO_3$, 25.0; dextrose, 9.7. The solution was continously aerated by a gas mixture of 95% O_2-5% CO_2. The right atrium was isolated and suspended in a tissue chamber containing 20 mL of PSS at 37°C, pH 7.4. Atria were allowed to equilibrate for 1 hr under a resting tension of 1 g.

The atrial rate and force were determined from contractions recorded by a Grass FT.03 force-displacement transducer and a Grass model 79 polygraph. The Basial atrial rate for control atria (as determined by counting the frequency of contractions) was 258 ± 7 bpm (n=29). Basal developed force of the spontaneously beating right atria was 0.33 ± 0.06 g (n=10). Dose-response curves for the peptides were obtained by cumulative addition of the respective peptides. Drug dose is calculated on the basis of the final concentration in the bath solution.

Example 4

Measurement of Intracellular Free Calcium Concentration *In Vitro*

Intracellular free calcium concentration was measured using the fluorescent dye FURA-2 according to the method of Grynkiewicz et al., J. Biol. Chem., 260, 3440 (1985) and Pang et al., P. N. A. S. (USA), 87, 623 (1990). UMR-106 rat osteosarcoma cells (ATCC CRL-1661) are incubated in 1-10 μM FURA-2 AM (Sigma Chemical Co., St. Louis), the acetomethoxy ester of FURA-2. Upon hydrolysis within the cell, FURA-2 is released which selectively binds to free Ca^{2+}. Binding to Ca^{2+} shifts the fluorescent spectrum of FURA-2. Quantitation is obtained by exciting the dye at two different wavelengths, preferably 340 and 380 nm and measuring the fluorescent emission at 510 nm. The concentration of calcium is proportional to the ratio of the fluorescence emitted at 340 nm to that at 380 nm.

KCl is used in the medium to stimulate Ca^{2+} uptake.

After the intracellular $[Ca^{2+}]_i$ had been measured, the cells were washed with the original medium and the analogues added and the intracellular $[Ca^{2+}]_i$ measured again. KCl was then added without washing to measure the effect of the analogue on KCl induced Ca^{2+} uptake. After measurement, the cells were washed with the medium 3-4 times and KCl again added to determine the recovery of the cells after removal of the analogue. Results are shown by actual traces and histograms summarizing the results. As can be seen from Figs. 10 a-d, PTH inhibits Ca^{2+} uptake as measured by the method. Figs. 11 a-c, 16, 29, 30, 33, 34, 38, 39, 40, 44 and 45-47 illustrate comparable results for the aa^{23} analogues.

The comparability of the analogues and PTH itself is considered to indicate that the analogues would be as useful as PTH for the treatment of osteoporosis.

Table I

Designation	Length	Source	Substitution	Site
Cs88	1-34	bovine	none	
Cs99	1-34	bovine	Ala	25
Cs100	1-34	bovine	Ala	26
Cs114	1-34	bovine	Ala	23
Cs117	1-34	bovine	Ala	27
Cs201	1-34	human	Asp	23
Cs205	1-34	human	Pro	23
Cs206	1-34	human	Asn	23
Cs207	1-34	human	Thr	23
Cs208	1-34	human	Ser	23
Cs209	1-34	human	Glu	23
Cs210	1-34	human	Gln	23
Cs211	1-34	human	Gly	23
Cs212	1-34	human	Cys	23
Cs213	1-34	human	Ile	23
Cs214	1-34	human	Lys	23
Cs215	1-34	human	His	23
Cs218	1-34	human	Tyr	23
Cs219	1-34	human	Phe	23
Cs220	1-34	human	Ala	23
Cs501	1-34	human	Met	23
Cs502	1-34	human	Leu	23
Cs503	1-34	human	Arg	23
Cs1001	1-34	human	none	
Cs2001	1-84	human	none	

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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JIE, Shan

(ii) TITLE OF INVENTION: PARATHYROID HORMONE ANALOGUES AS OSTEOPOROTIC CONTROL AGENTS

(iii) NUMBER OF SEQUENCES: 36

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser
1               5                   10                  15

Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His
            20                  25                  30

Asn Phe Val Ala Leu Gly Ala Ser Ile Ala Tyr Arg Asp Gly Ser Ser
        35                  40                  45

Gln Arg Pro Arg Lys Lys Glu Asp Asn Val Leu Val Glu Ser His Gln
    50                  55                  60

Lys Ser Leu Gly Glu Ala Asp Lys Ala Asp Val Asp Val Leu Ile Lys
65                  70                  75                  80

Ala Lys Pro Gln
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1               5                   10                  15

Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His
            20                  25                  30

Asn Phe Val Ala Leu Gly Ala Ser Ile Ala Tyr Arg Asp Gly Ser Ser
        35                  40                  45

Gln Arg Pro Arg Lys Lys Glu Asp Asn Val Leu Val Glu Ser His Gln
    50                  55                  60

Lys Ser Leu Gly Glu Ala Asp Lys Ala Asp Val Asp Val Leu Ile Lys
65                  70                  75                  80

Ala Lys Pro Gln
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser
1 5 10 15

Ser Met Glu Arg Val Glu Xaa Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser
1 5 10 15

Ser Met Glu Arg Val Glu Ala Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser
1 5 10 15

Ser Met Glu Arg Val Glu Arg Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser
1 5 10 15

Ser Met Glu Arg Val Glu Asn Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser
1 5 10 15

Ser Met Glu Arg Val Glu Asp Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser
1 5 10 15

Ser Met Glu Arg Val Glu Cys Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser
1 5 10 15

Ser Met Glu Arg Val Glu Gln Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser
1 5 10 15

Ser Met Glu Arg Val Glu Glu Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser
1 5 10 15

Ser Met Glu Arg Val Glu Gly Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser
1 5 10 15

Ser Met Glu Arg Val Glu His Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser
1 5 10 15

Ser Met Glu Arg Val Glu Ile Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser
1 5 10 15

Ser Met Glu Arg Val Glu Lys Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser
1 5 10 15

Ser Met Glu Arg Val Glu Met Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser
1 5 10 15

Ser Met Glu Arg Val Glu Pro Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser
1 5 10 15

Ser Met Glu Arg Val Glu Ser Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser
1               5                   10                  15

Ser Met Glu Arg Val Glu Thr Leu Arg Lys Lys Leu Gln Asp Val His
            20                  25                  30

Asn Phe
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1               5                   10                  15

Ser Met Glu Arg Val Glu Xaa Leu Arg Lys Lys Leu Gln Asp Val His
            20                  25                  30

Asn Phe
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1 5 10 15

Ser Met Glu Arg Val Glu Ala Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1 5 10 15

Ser Met Glu Arg Val Glu Arg Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1 5 10 15

Ser Met Glu Arg Val Glu Asn Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1               5                   10                  15

Ser Met Glu Arg Val Glu Asp Leu Arg Lys Lys Leu Gln Asp Val His
            20                  25                  30

Asn Phe
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1               5                   10                  15

Ser Met Glu Arg Val Glu Cys Leu Arg Lys Lys Leu Gln Asp Val His
            20                  25                  30

Asn Phe
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1 5 10 15

Ser Met Glu Arg Val Glu Gln Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1 5 10 15

Ser Met Glu Arg Val Glu Glu Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1 5 10 15

Ser Met Glu Arg Val Glu Gly Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1               5                   10                  15

Ser Met Glu Arg Val Glu His Leu Arg Lys Lys Leu Gln Asp Val His
            20                  25                  30

Asn Phe
```

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1               5                   10                  15

Ser Met Glu Arg Val Glu Ile Leu Arg Lys Lys Leu Gln Asp Val His
            20                  25                  30

Asn Phe
```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1 5 10 15

Ser Met Glu Arg Val Glu Lys Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1 5 10 15

Ser Met Glu Arg Val Glu Met Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1 5 10 15

Ser Met Glu Arg Val Glu Pro Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1 5 10 15

Ser Met Glu Arg Val Glu Ser Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1 5 10 15

Ser Met Glu Arg Val Glu Thr Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser
1   5   10   15

Ser Met Glu Arg Val Glu Xaa Leu Arg Lys Lys Leu Gln Asp Val His
            20   25   30

Asn Phe Val Ala Leu Gly Ala Ser Ile Ala Tyr Arg Asp Gly Ser Ser
        35   40   45

Gln Arg Pro Arg Lys Lys Glu Asp Asn Val Leu Val Glu Ser His Gln
    50   55   60

Lys Ser Leu Gly Glu Ala Asp Lys Ala Asp Val Asp Val Leu Ile Lys
65   70   75   80

Ala Lys Pro Gln

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1   5   10   15

Ser Met Glu Arg Val Glu Xaa Leu Arg Lys Lys Leu Gln Asp Val His
            20   25   30

Asn Phe Val Ala Leu Gly Ala Ser Ile Ala Tyr Arg Asp Gly Ser Ser
        35   40   45

Gln Arg Pro Arg Lys Lys Glu Asp Asn Val Leu Val Glu Ser His Gln
    50   55   60

Lys Ser Leu Gly Glu Ala Asp Lys Ala Asp Val Asp Val Leu Ile Lys
65   70   75   80

Ala Lys Pro Gln

CLAIMS

1. A bovine parathyroid hormone analogue comprising the structure shown in SEQ ID NO:3, wherein Xaa is Alanine (Ala), Arginine (Arg), Asparagine (Asn), Aspartic acid (Asp), Cysteine (Cys), Glutamine (Gln), Glutamic acid (Glu), Glycine (Gly), Histidine (His), Isoleucine (Ile), Lysine (Lys), Methionine (Met), Proline (Pro), Serine (Ser) or Threonine (Thr).

2. The bovine parathyroid hormone analogue having the structure shown in SEQ ID NO:4.

3. The bovine parathyroid hormone analogue having the structure shown in SEQ ID NO:5.

4. The bovine parathyroid hormone analogue having the structure shown in SEQ ID NO:6.

5. The bovine parathyroid hormone analogue having the structure shown in SEQ ID NO:7.

6. The bovine parathyroid hormone analogue having the structure shown in SEQ ID NO:8.

7. The bovine parathyroid hormone analogue having the structure shown in SEQ ID NO:9.

8. The bovine parathyroid hormone analogue having the structure shown in SEQ ID NO:10.

9. The bovine parathyroid hormone analogue having the structure shown in SEQ ID NO:11.

10. The bovine parathyroid hormone analogue having the structure shown in SEQ ID NO:12.

11. The bovine parathyroid hormone analogue having the structure shown in SEQ ID NO:13.

12. The bovine parathyroid hormone analogue having the structure shown in SEQ ID NO:14.

13. The bovine parathyroid hormone analogue having the structure shown in SEQ ID NO:15.

14. The bovine parathyroid hormone analogue having the structure shown in SEQ ID NO:16.

15. The bovine parathyroid hormone analogue having the structure shown in SEQ ID NO:17.

16. The bovine parathyroid hormone analogue having the structure shown in SEQ ID NO:18.

17. A human parathyroid hormone analogue comprising the structure shown in SEQ ID NO:19, wherein Xaa is Alanine (Ala), Arginine (Arg), Asparagine (Asn), Aspartic acid (Asp), Cysteine (Cys), Glutamine (Gln), Glutamic acid (Glu), Glycine (Gly), Histidine (His), Isoleucine (Ile), Lysine (Lys), Methionine (Met), Proline (Pro), Serine (Ser) or Threonine (Thr).

18. The human parathyroid hormone analogue having the structure shown in SEQ ID NO:20.

19. The human parathyroid hormone analogue having the structure shown in SEQ ID NO:21.

20. The human parathyroid hormone analogue having the structure shown in SEQ ID NO:22.

21. The human parathyroid hormone analogue having the structure shown in SEQ ID NO:23.

22. The human parathyroid hormone analogue having the structure shown in SEQ ID NO:24.

23. The human parathyroid hormone analogue having the structure shown in SEQ ID NO:25.

24. The human parathyroid hormone analogue having the structure shown in SEQ ID NO:26.

25. The human parathyroid hormone analogue having the structure shown in SEQ ID NO:27.

26. The human parathyroid hormone analogue having the structure shown in SEQ ID NO:28.

27. The human parathyroid hormone analogue having the structure shown in SEQ ID NO:29.

28. The human parathyroid hormone analogue having the structure shown in SEQ ID NO:30.

29. The human parathyroid hormone analogue having the structure shown in SEQ ID NO:31.

30. The human parathyroid hormone analogue having the structure shown in SEQ ID NO:32.

31. The human parathyroid hormone analogue having the structure shown in SEQ ID NO:33.

32. The human parathyroid hormone analogue having the structure shown in SEQ ID NO:34.

33. A bovine parathyroid hormone analogue comprising the structure shown in SEQ ID NO:35, wherein Xaa is Alanine (Ala), Arginine (Arg), Asparagine (Asn), Aspartic acid (Asp), Cysteine (Cys), Glutamine (Gln), Glutamic acid (Glu), Glycine (Gly),

Histidine (His), Isoleucine (Ile), Lysine (Lys), Methionine (Met), Proline (Pro), Serine (Ser) or Threonine (Thr).

34. A human parathyroid hormone analogue comprising the structure shown in SEQ ID NO:36, wherein Xaa is Alanine (Ala), Arginine (Arg), Asparagine (Asn), Aspartic acid (Asp), Cysteine (Cys), Glutamine (Gln), Glutamic acid (Glu), Glycine (Gly), Histidine (His), Isoleucine (Ile), Lysine (Lys), Methionine (Met), Proline (Pro), Serine (Ser) or Threonine (Thr).

35. A pharmaceutical composition comprising a PTH analogue according to any one of claims 1-34 and a pharmaceutically acceptable carrier.

36. A method of treatment of osteoporosis in a patient in need of such treatment without causing substantial induction of hypotension, smooth muscle relaxation and cardiac inotropic and chronotropic action, said method comprising administering an osteoporotic effective amount of a PTH analogue according to any one of claims 1-34.

Fig. 1a

H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-
Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-
Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-Val-Ala-Leu-Gly-
Ala-Ser-Ile-Ala-Tyr-Arg-Asp-Gly-Ser-Ser-Gln-Arg-Pro-
Arg-Lys-Lys-Glu-Asp-Asn-Val-Leu-Val-Glu-Ser-His-Gln-
Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asp-Val-
Leu-Ile-Lys-Ala-Lys-Pro-Gln-CO_2H

Fig. 1b

H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-
Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-
Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-Val-Ala-Leu-Gly-
Ala-Ser-Ile-Ala-Tyr-Arg-Asp-Gly-Ser-Ser-Gln-Arg-Pro-
Arg-Lys-Lys-Glu-Asp-Asn-Val-Leu-Val-Glu-Ser-His-Gln-
Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asp-Val-
Leu-Ile-Lys-Ala-Lys-Pro-Gln-CO_2H

Fig. 2a

H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Xaa-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 2b

H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Ala-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 2c

H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Arg-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 2d

H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Asn-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 2e

H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Asp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 2f

H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Cys-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 2g

H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Gln-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 2h

H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Glu-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 2i

H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Gly-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 2j

H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-His-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 2k

H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Ile-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 2l

H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Lys-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 2m

H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Met-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 2n

H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Pro-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 2o

H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Ser-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 2p

H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Thr-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 3a

H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Xaa-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 3b

H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Ala-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 3c

H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Arg-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 3d

H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Asp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 3e

H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Asn-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 3f

H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Cys-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 3g

H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Gln-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 3h

H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Glu-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 3i

H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-
Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Gly-Leu-Arg-
Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 3j

H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-
Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-His-Leu-Arg-
Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 3k

H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-
Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Ile-Leu-Arg-
Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 3l

H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-
Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Lys-Leu-Arg-
Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 3m

H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-
Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Met-Leu-Arg-
Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 3n

H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-
Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Pro-Leu-Arg-
Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 3o

H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-
Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Ser-Leu-Arg-
Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 3p

H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-
Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Thr-Leu-Arg-
Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-CO_2H

Fig. 4

H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-
Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Xaa-Leu-Arg-
Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-Val-Ala-Leu-Gly-
Ala-Ser-Ile-Ala-Tyr-Arg-Asp-Gly-Ser-Ser-Gln-Arg-Pro-
Arg-Lys-Lys-Glu-Asp-Asn-Val-Leu-Val-Glu-Ser-His-Gln-
Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asp-Val-
Leu-Ile-Lys-Ala-Lys-Pro-Gln-CO_2H

Fig. 5

H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-
Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Xaa-Leu-Arg-
Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-Val-Ala-Leu-Gly-
Ala-Ser-Ile-Ala-Tyr-Arg-Asp-Gly-Ser-Ser-Gln-Arg-Pro-
Arg-Lys-Lys-Glu-Asp-Asn-Val-Leu-Val-Glu-Ser-His-Gln-
Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asp-Val-
Leu-Ile-Lys-Ala-Lys-Pro-Gln-CO_2H

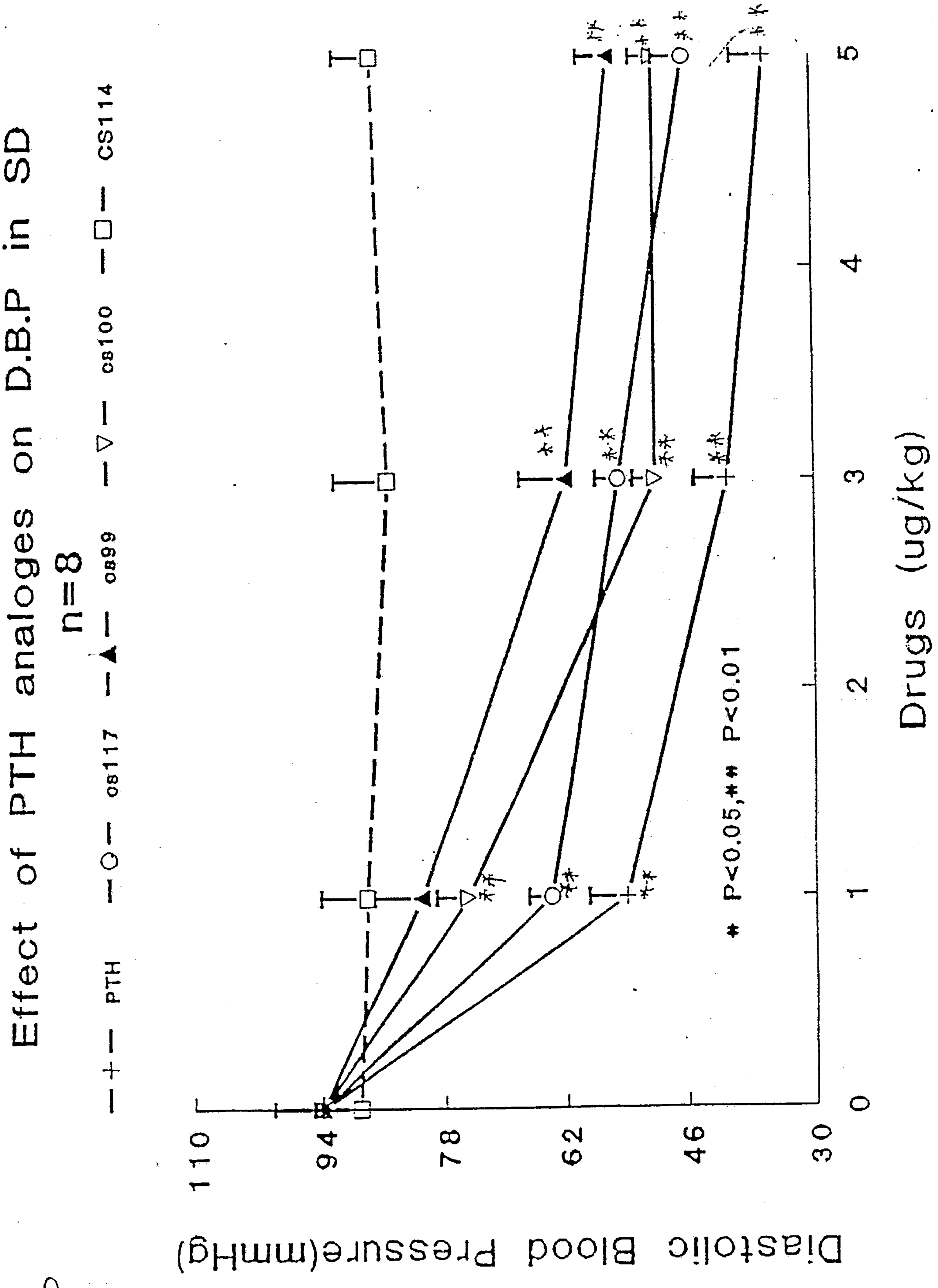
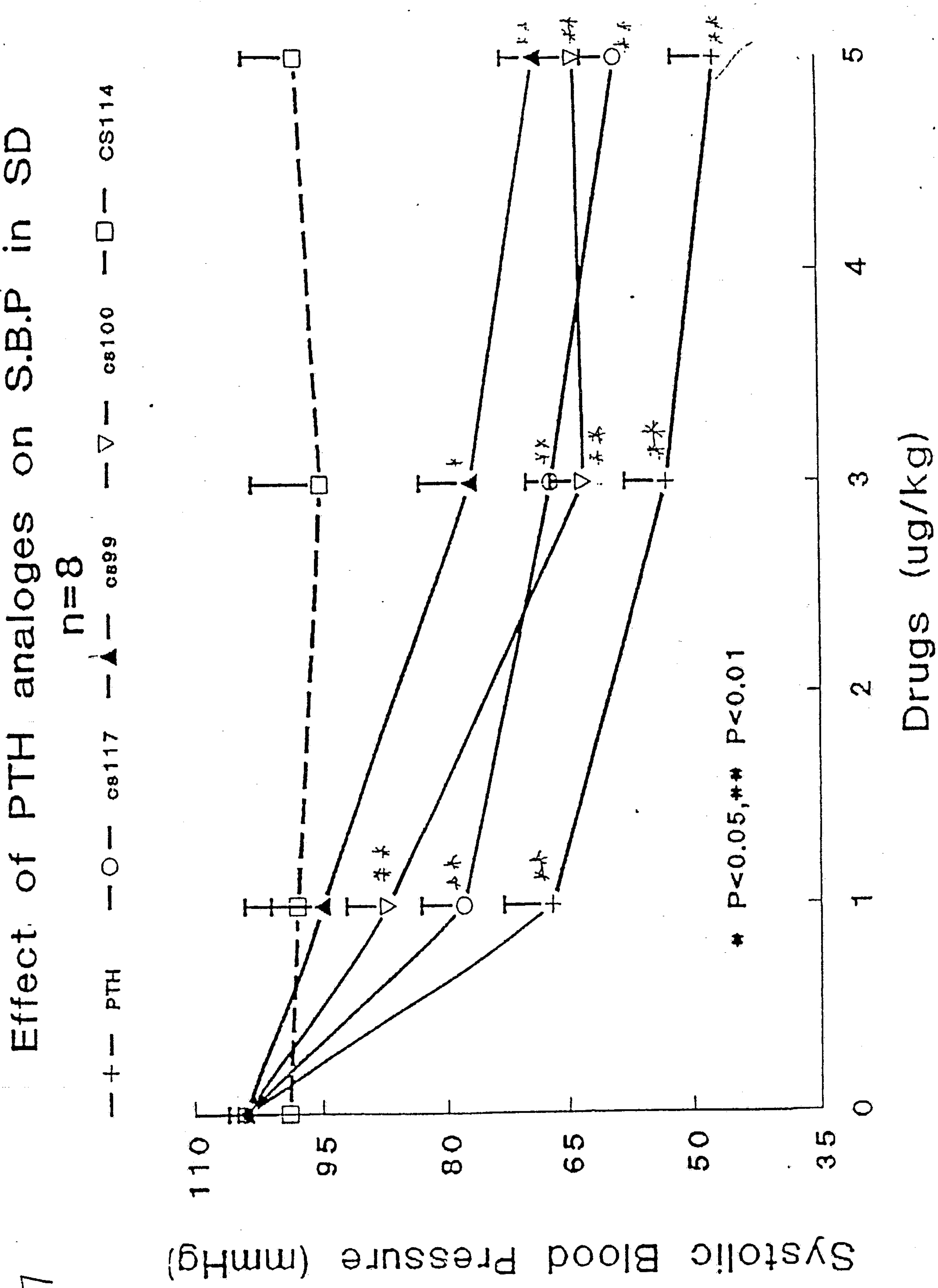

Effect of PTH analoges on D.B.P in SD
n=8
PTH
cs117
cs99
cs100
CS114
Diastolic Blood Pressure(mmHg)
110
94
78
62
46
30
0
1
2
3
4
5
Drugs (ug/kg)
* P<0.05,** P<0.01

Fig. 6



Effect of PTH analoges on S.B.P in SD
n=8
PTH
cs117
cs99
cs100
CS114
Systolic Blood Pressure (mmHg)
110
95
80
65
50
35
0
1
2
3
4
5
Drugs (ug/kg)
* P<0.05,** P<0.01

Fig 7

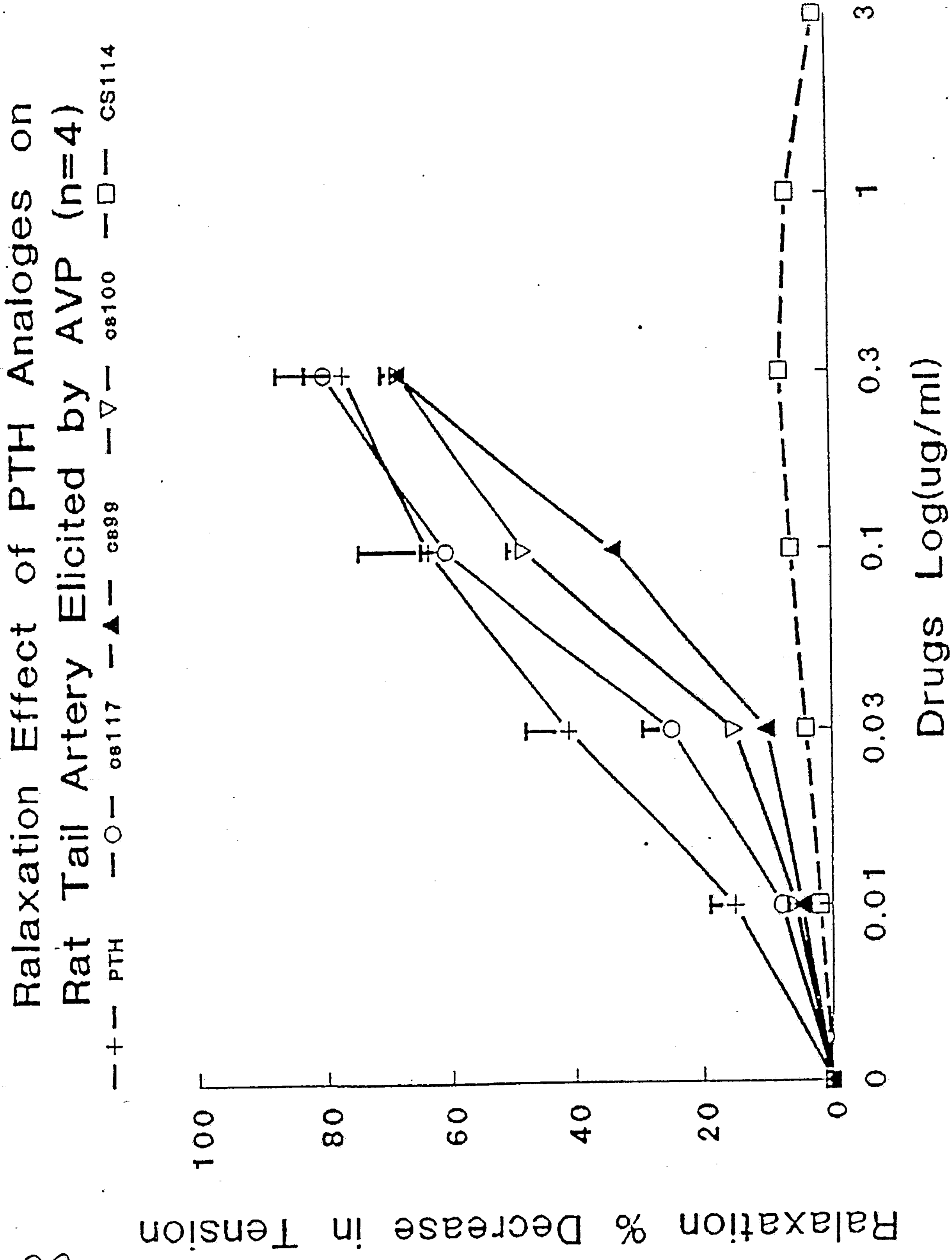

Ralaxation Effect of PTH Analoges on
Rat Tail Artery Elicited by AVP (n=4)
PTH
cs117
cs99
cs100
CS114
Ralaxation % Decrease in Tension
100
80
60
40
20
0
0
0.01
0.03
0.1
0.3
1
3
Drugs Log(ug/ml)

Fig. 8

Fig. 9

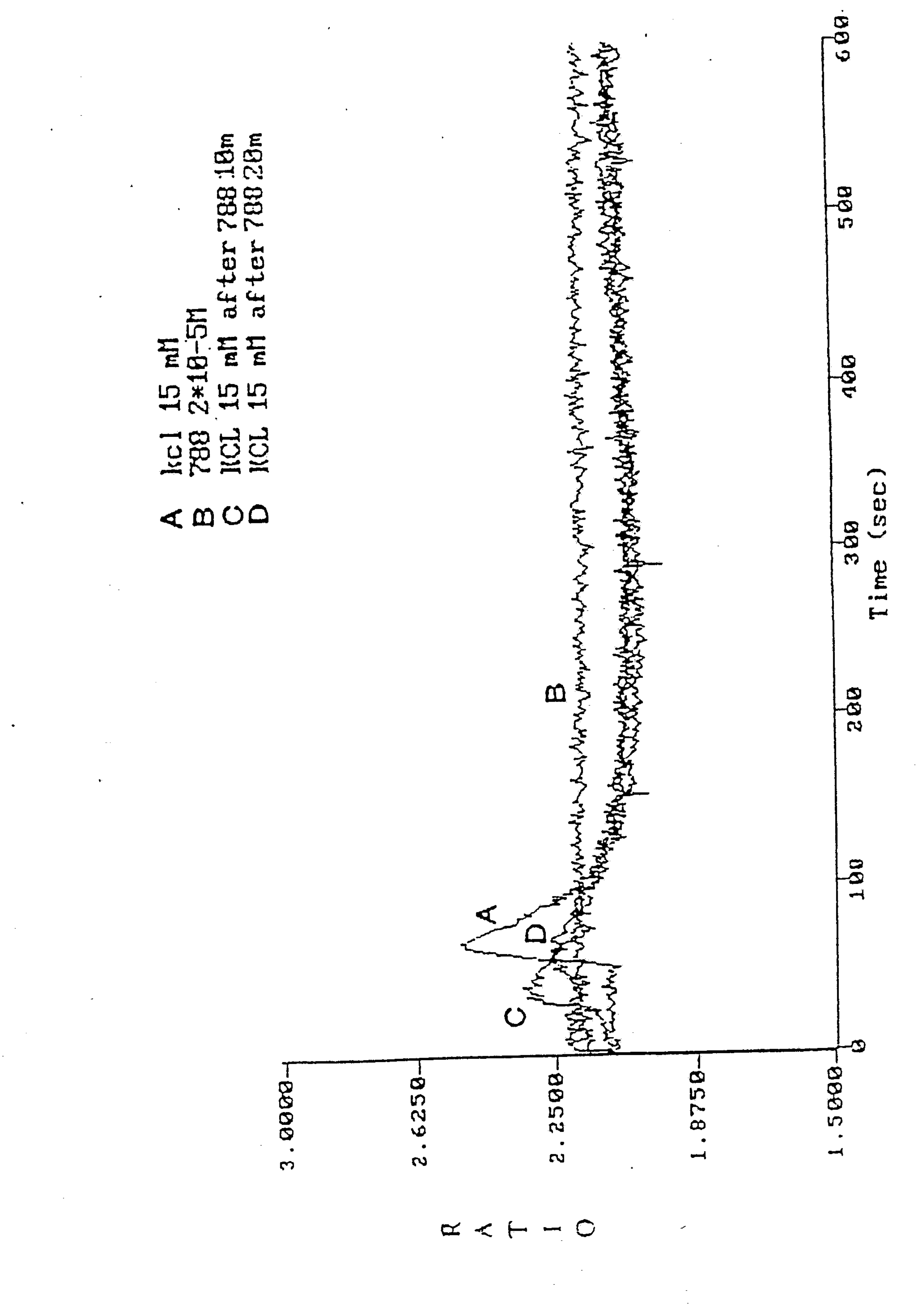

A kcl 15 mM
B 788 2*10-5M
C KCL 15 mM after 788 10m
D KCL 15 mM after 788 20m
A
B
C
D
RATIO
3.0000
2.6250
2.2500
1.8750
1.5000
0
100
200
300
400
500
600
Time (sec)

Fig 10a

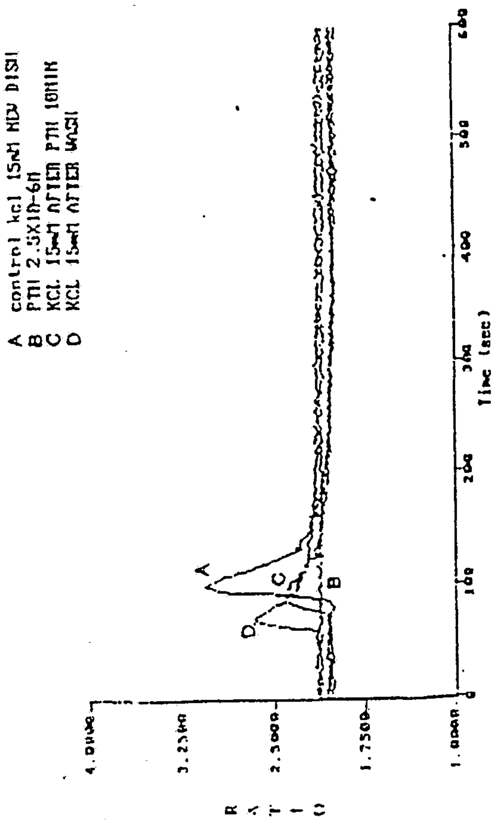


Fig. 10b

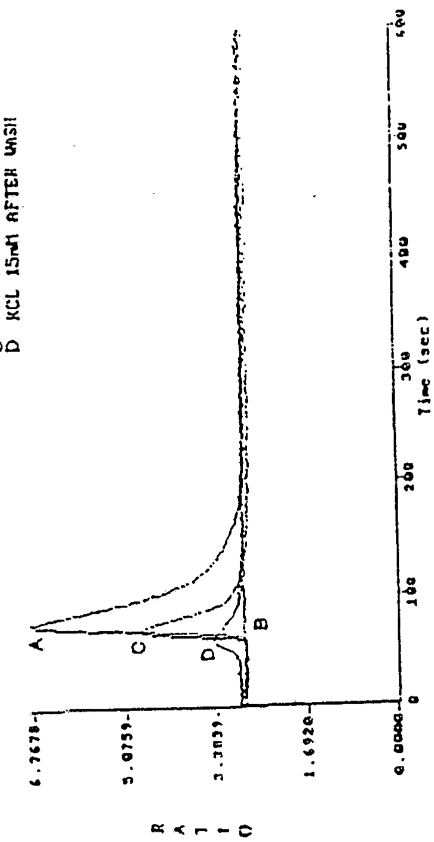


Fig 10c

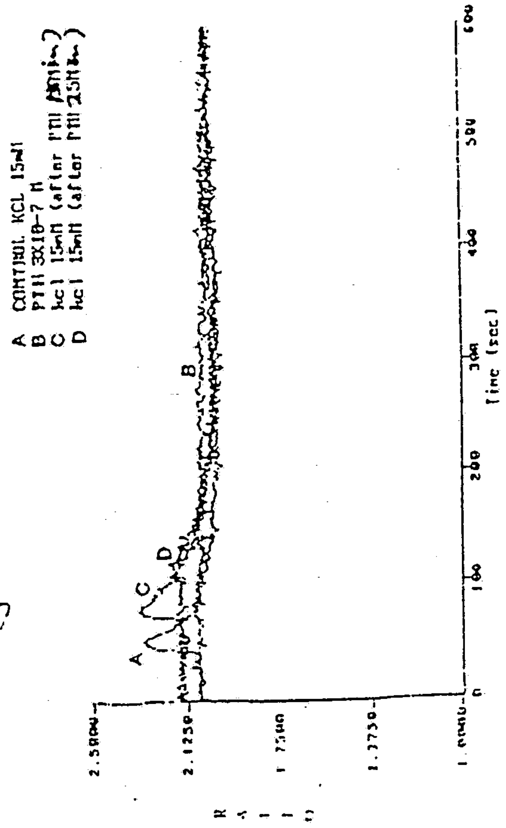


Fig 10d

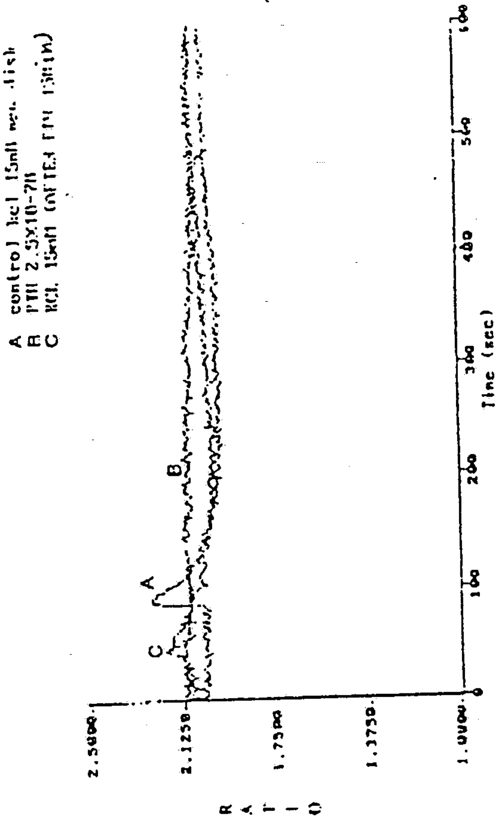


Fig. 11a

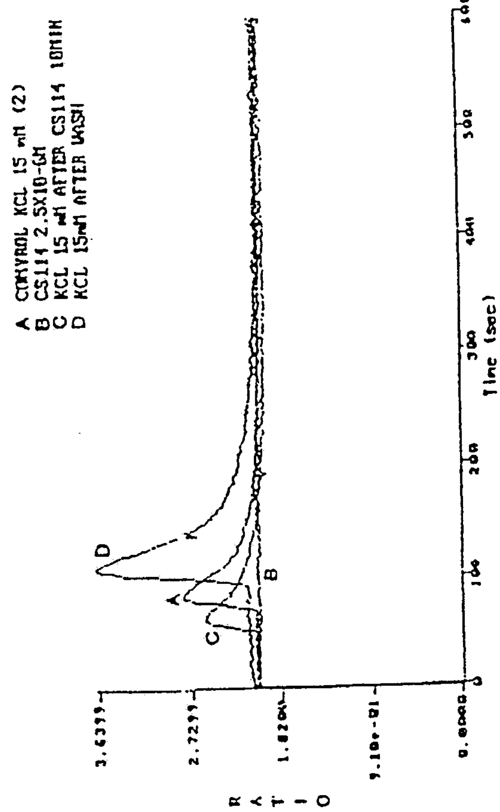


Fig. 11c

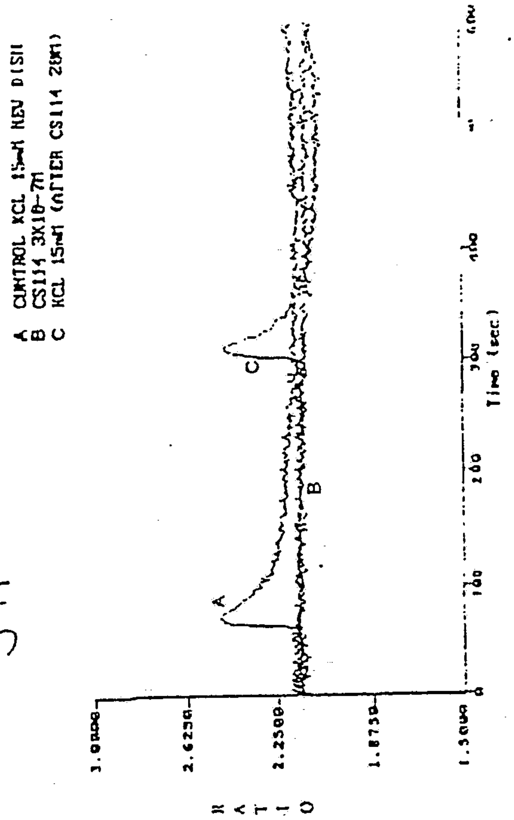
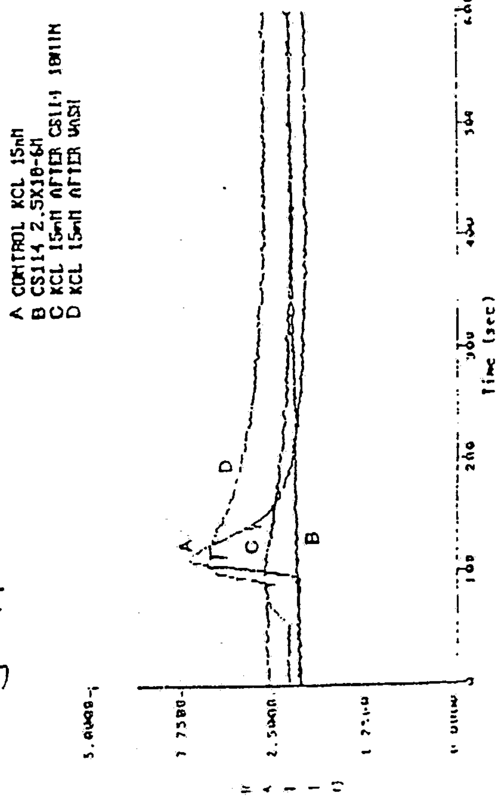


Fig. 11b



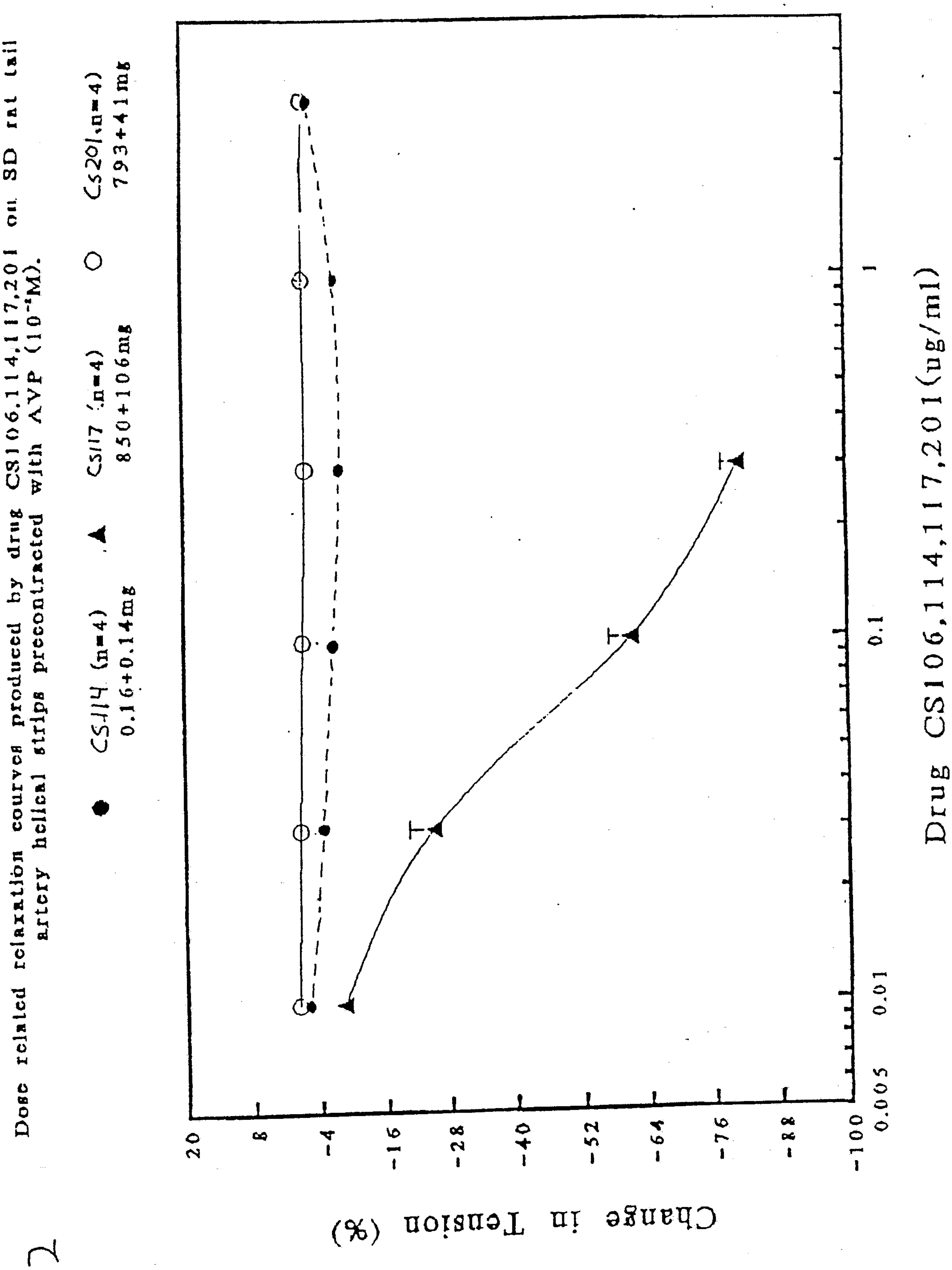

Dose related relaxation courves produced by drug CS106,114,117,201 on SD rat tail artery helical strips precontracted with AVP (10⁻⁷M).
CS114 (n=4) 0.16+0.14mg
CS117 (n=4) 850+106mg
CS201 (n=4) 793+41mg
20
8
-4
-16
-28
-40
-52
-64
-76
-88
-100
0.005
0.01
0.1
1
Change in Tension (%)
Drug CS106,114,117,201(ug/ml)

Fig 12

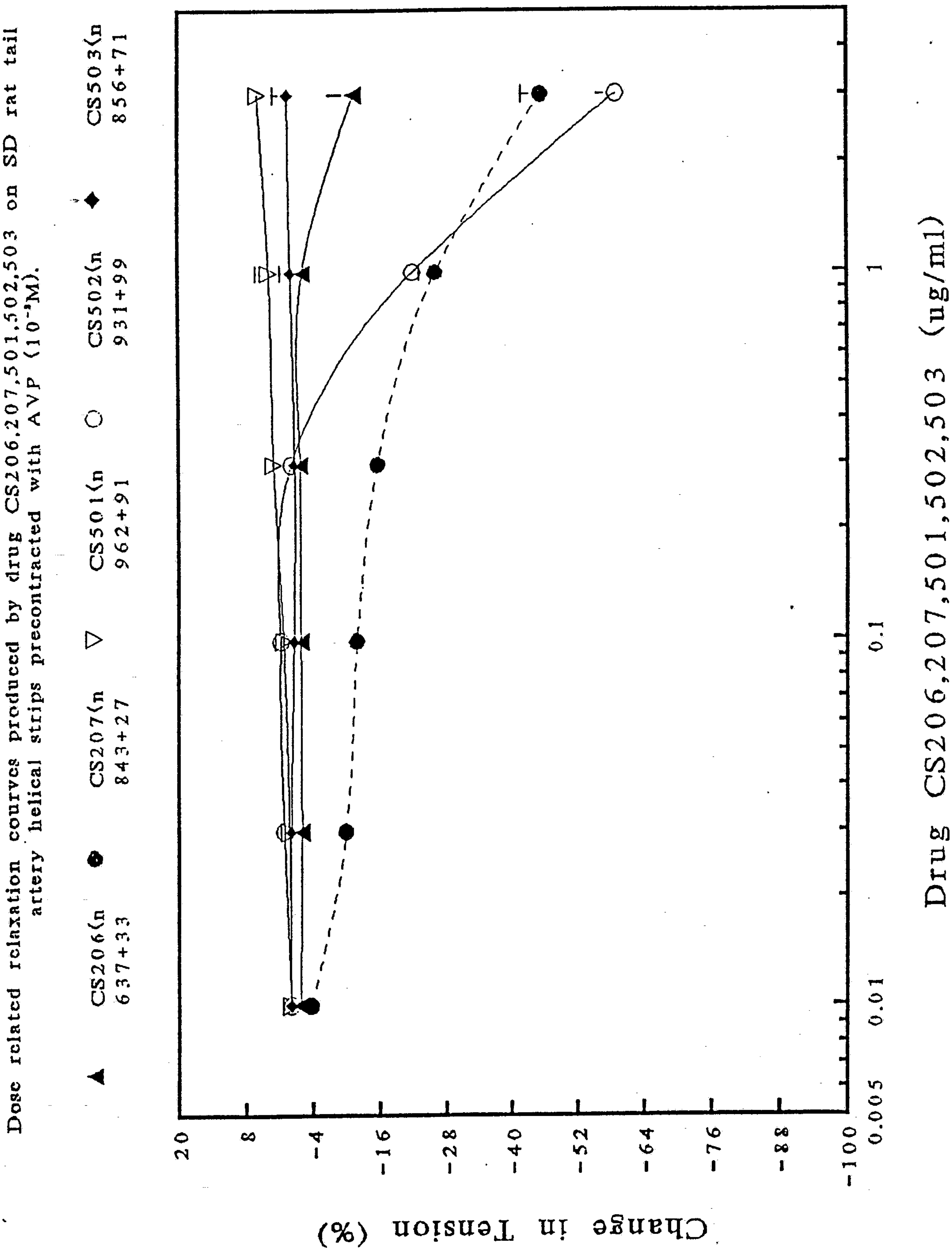

Dose related relaxation courves produced by drug CS206,207,501,502,503 on SD rat tail artery helical strips precontracted with AVP (10⁻⁸M).
CS206(n 637+33
CS207(n 843+27
CS501(n 962+91
CS502(n 931+99
CS503(n 856+71
Change in Tension (%)
20
8
-4
-16
-28
-40
-52
-64
-76
-88
-100
0.005
0.01
0.1
1
Drug CS206,207,501,502,503 (ug/ml)

Fig. 13

Fig. 14

Dose related relaxation curves produced by drug CS117,201,501,502 and 503 on SD rat tail artery helical strips precontracted with KCL (60mM)

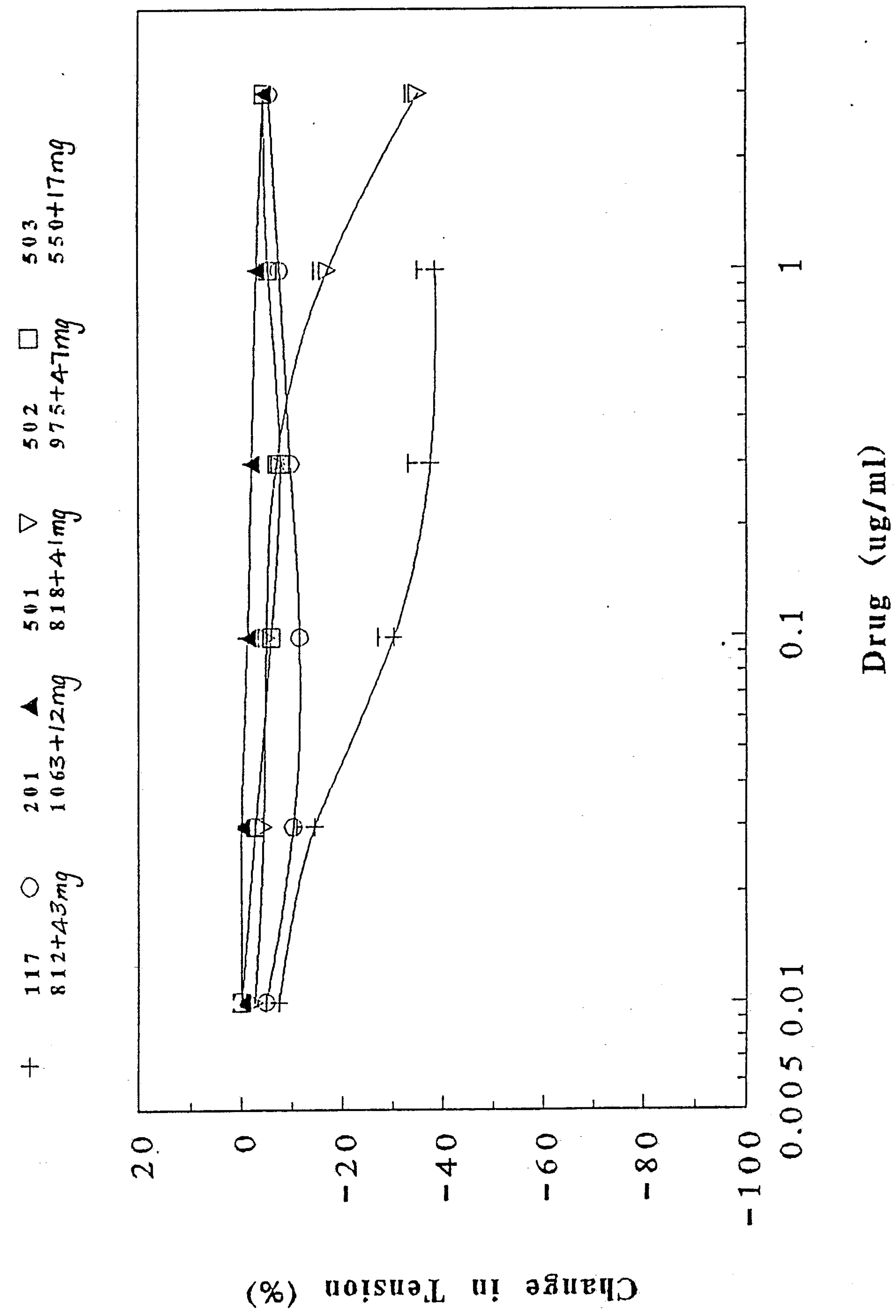
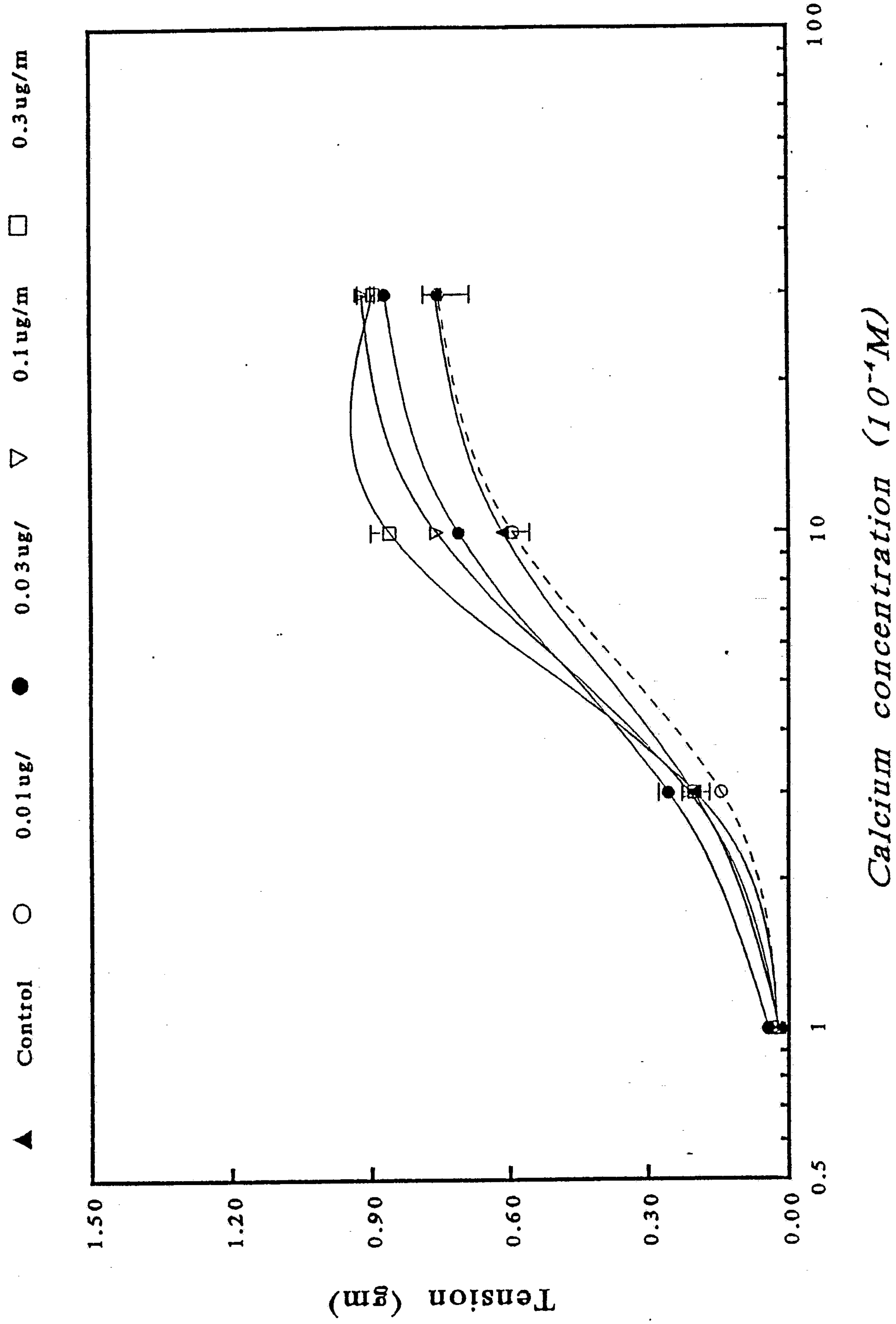


Fig. 15

Dose related inhibition of extracellular calcium influx by drug CS114
The rat tail artery helical strips were precontracted with NE(10-7)M



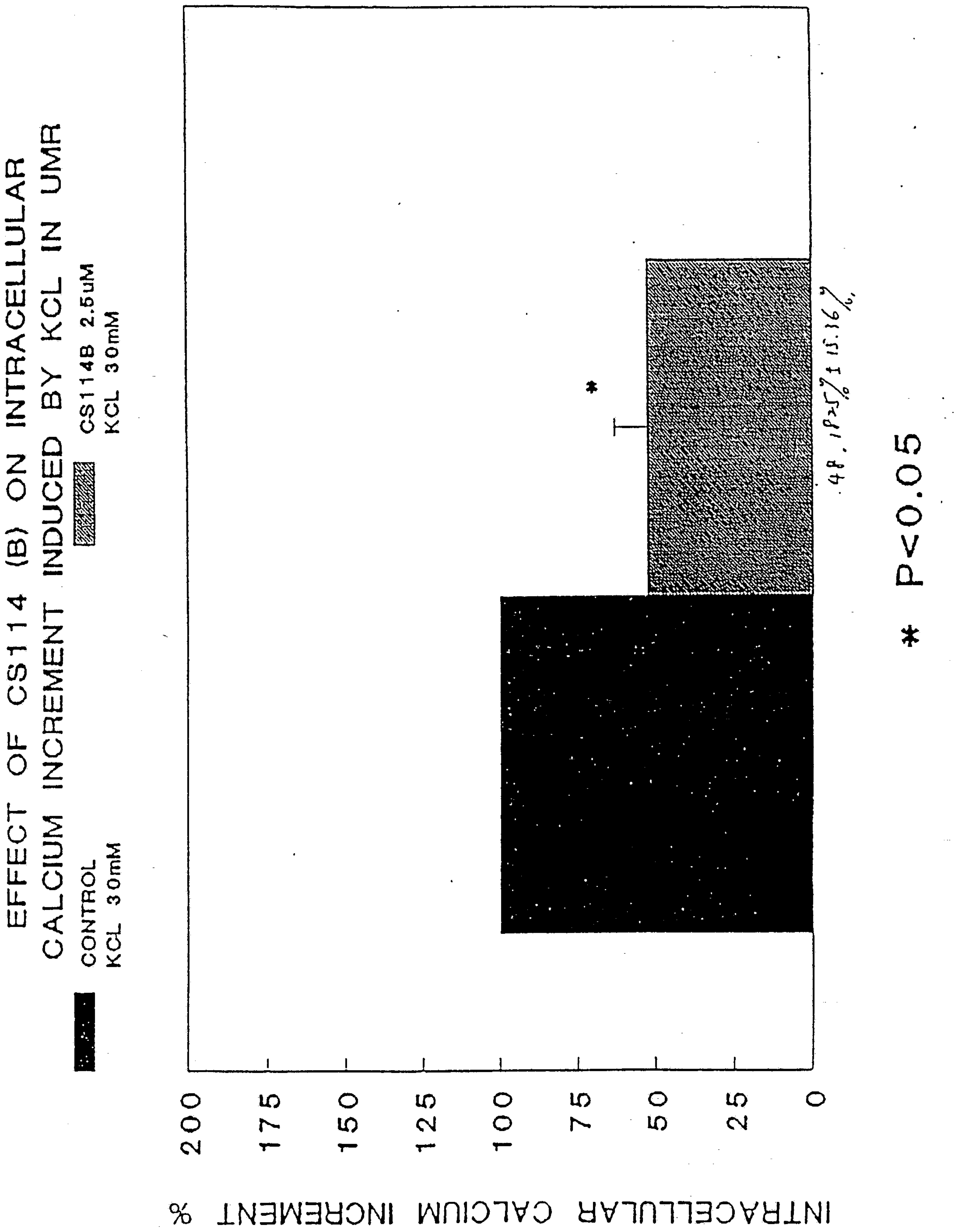

EFFECT OF CS114 (B) ON INTRACELLULAR
CALCIUM INCREMENT INDUCED BY KCL IN UMR
CONTROL
KCL 30mM
CS114B 2.5uM
KCL 30mM
INTRACELLULAR CALCIUM INCREMENT %
200
175
150
125
100
75
50
25
0
*
* P<0.05

Fig. 16

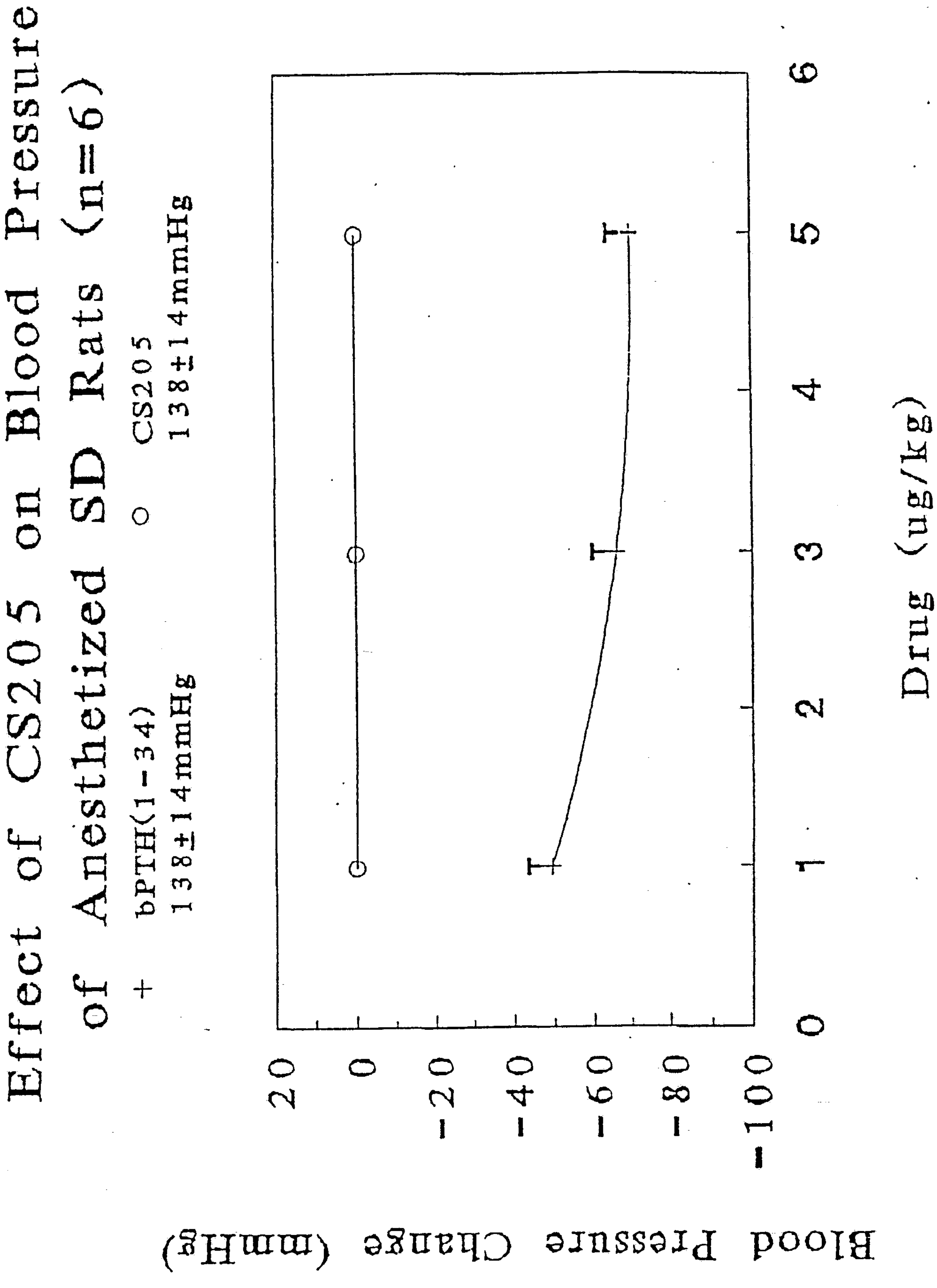

Effect of CS205 on Blood Pressure of Anesthetized SD Rats (n=6)
+ bPTH(1-34) 138±14mmHg
○ CS205 138±14mmHg
Blood Pressure Change (mmHg)
20
0
-20
-40
-60
-80
-100
0
1
2
3
4
5
6
Drug (ug/kg)

Fig. 17

Fig. 18

Dose related relaxation curves produced by drug CS205 on SD rat tail artery helical strips precontracted with KCL (60mM), NE (10^{-6}M) or AVP (10^{-1}M)

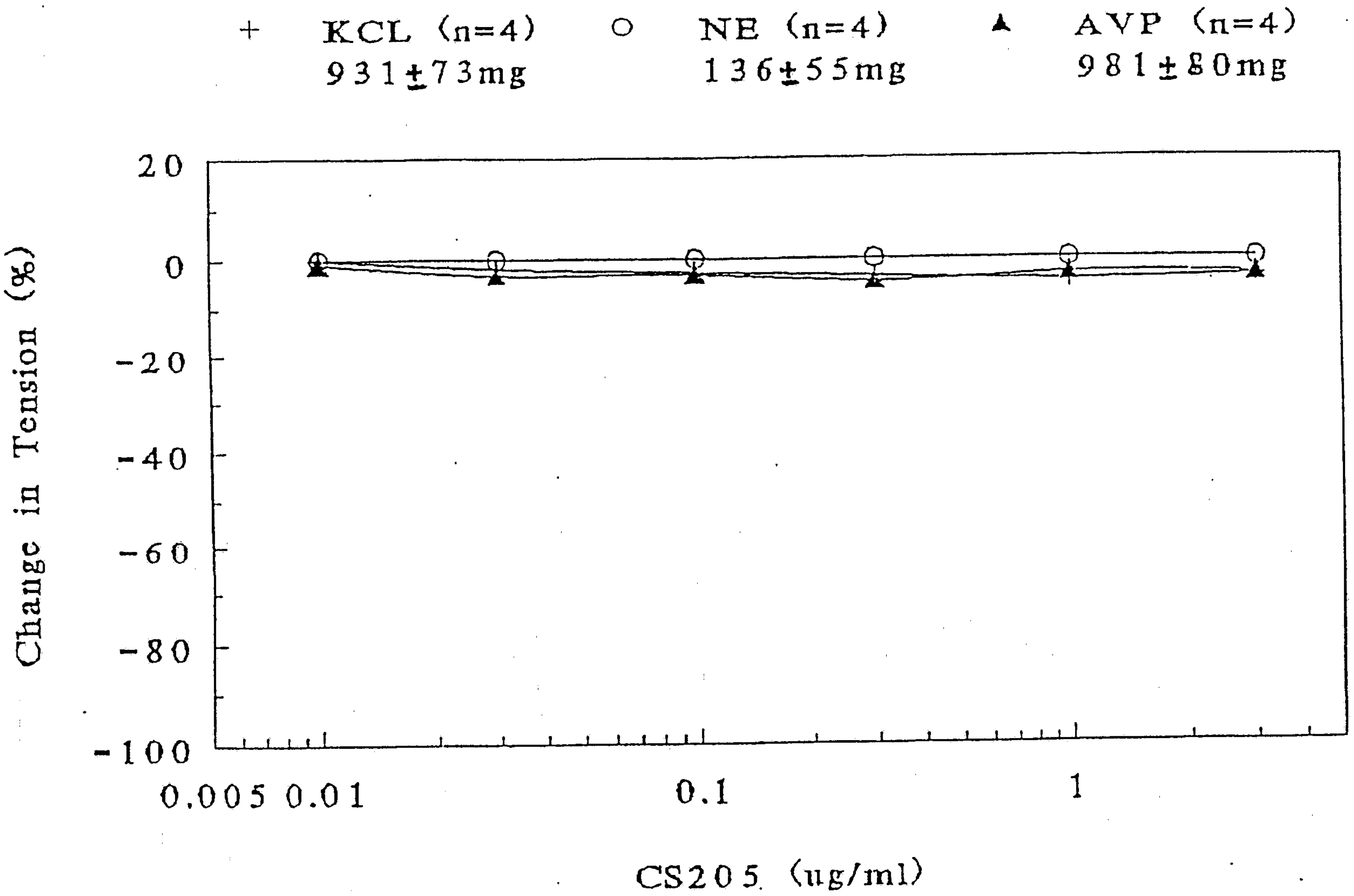
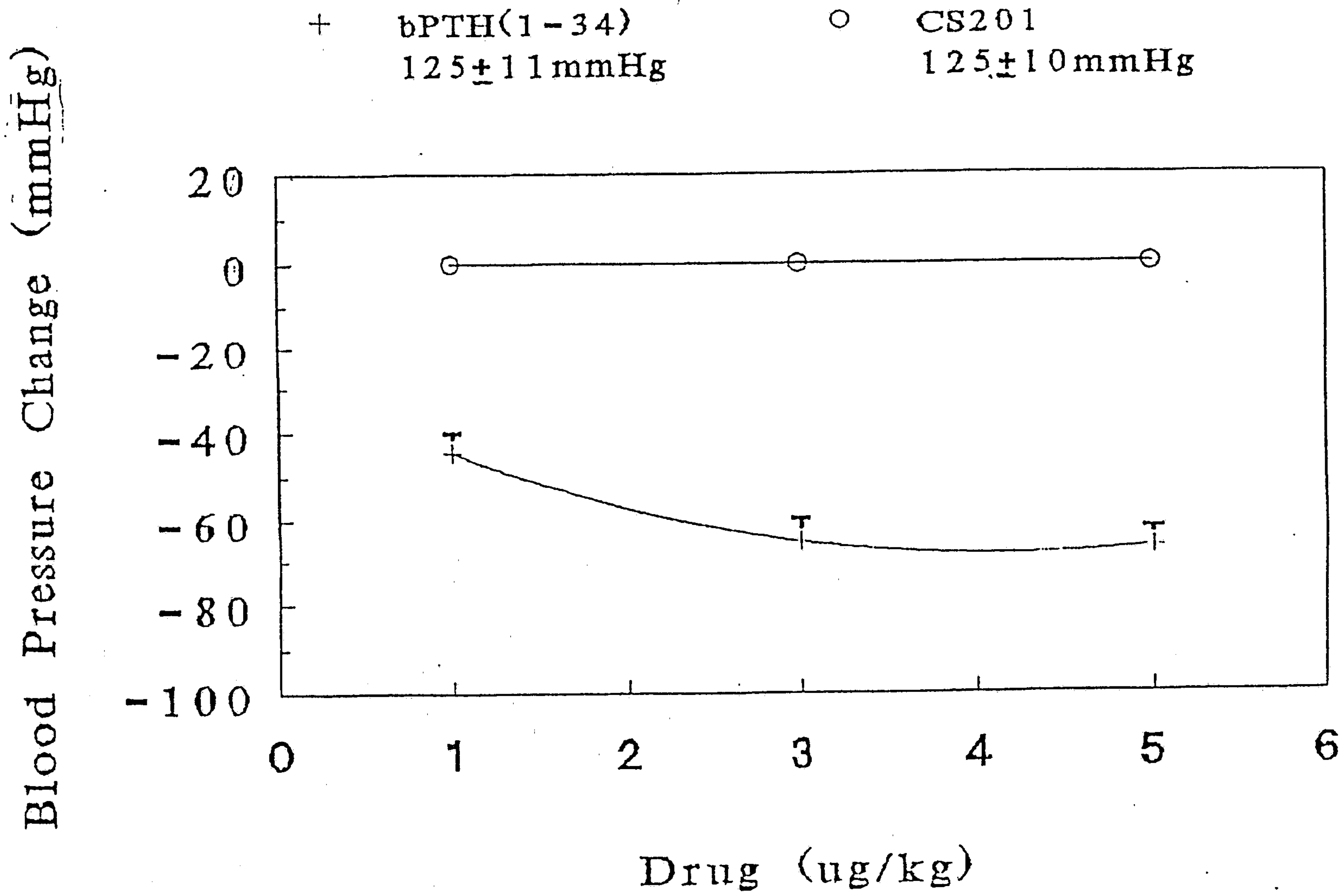


Fig. 19

Effect of CS201 on Blood Pressure of Anesthetized SD Rats (n=6)



Dose related relaxation curves procuced by drug CS201 on SD rat tail artery helical strips precontracted with KCL (60mM), NE ($3x10^{-7}M$) or AVP ($10^{-9}M$)

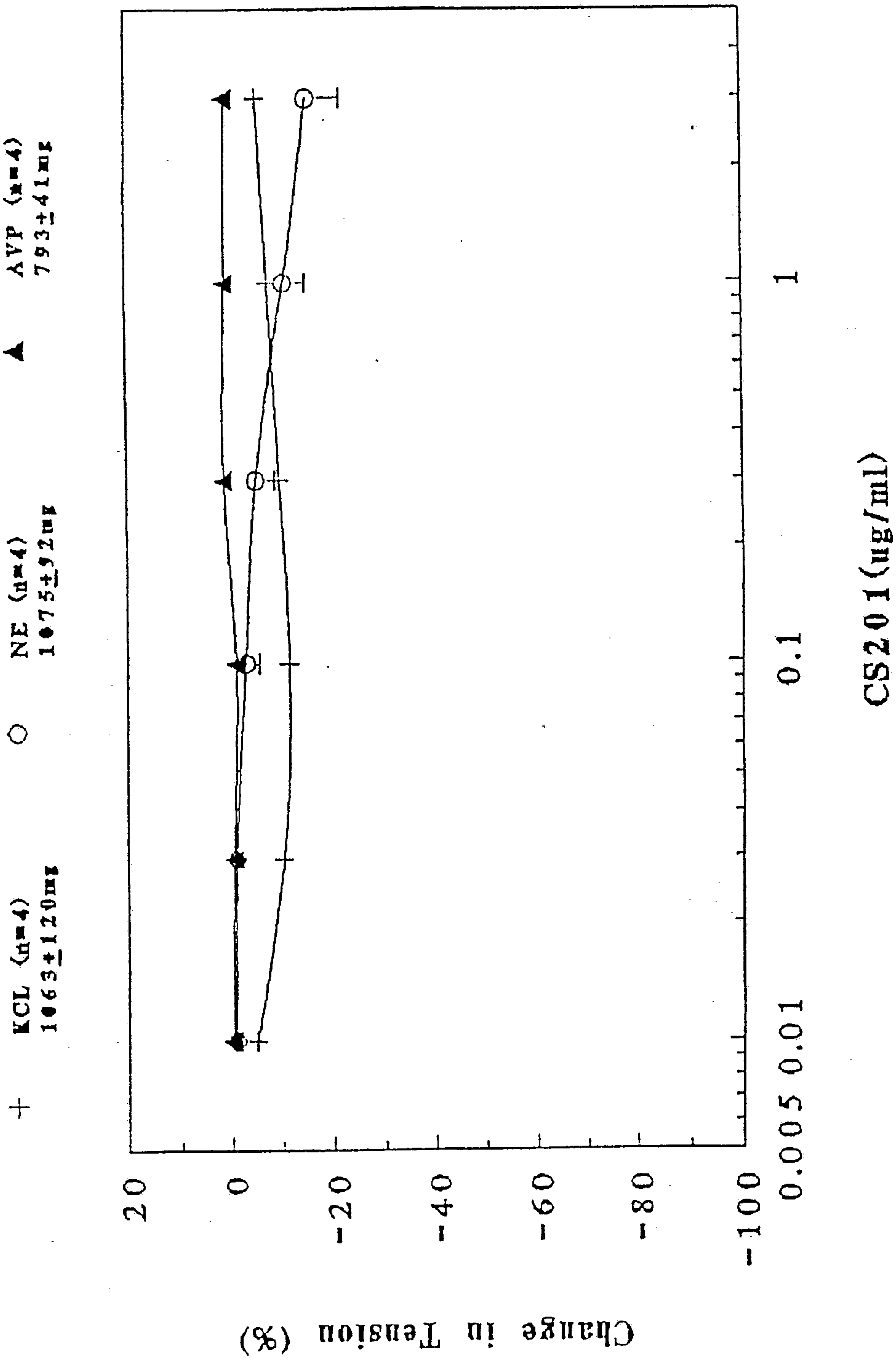
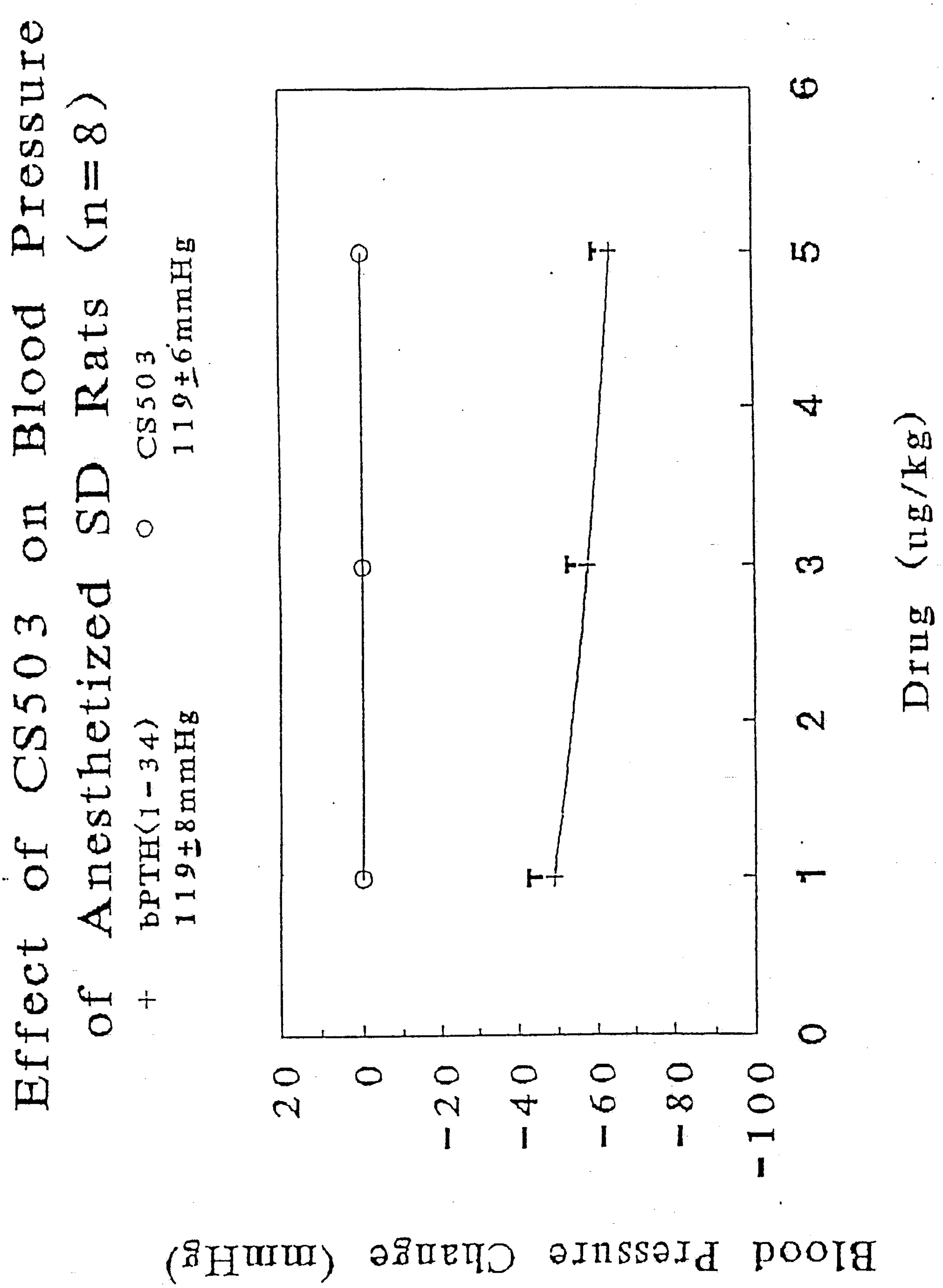


Fig. 20



Effect of CS503 on Blood Pressure
of Anesthetized SD Rats (n=8)
+ bPTH(1-34) 119±8mmHg
○ CS503 119±6mmHg
Blood Pressure Change (mmHg)
20
0
-20
-40
-60
-80
-100
Drug (ug/kg)
0
1
2
3
4
5
6

Fig 21

Dose related relaxation curves produced by drug CS503 on SD rat tail artery helical strips precontracted with KCL (60mM), NE (10^{-6}M) or AVP (10^{-8}M)

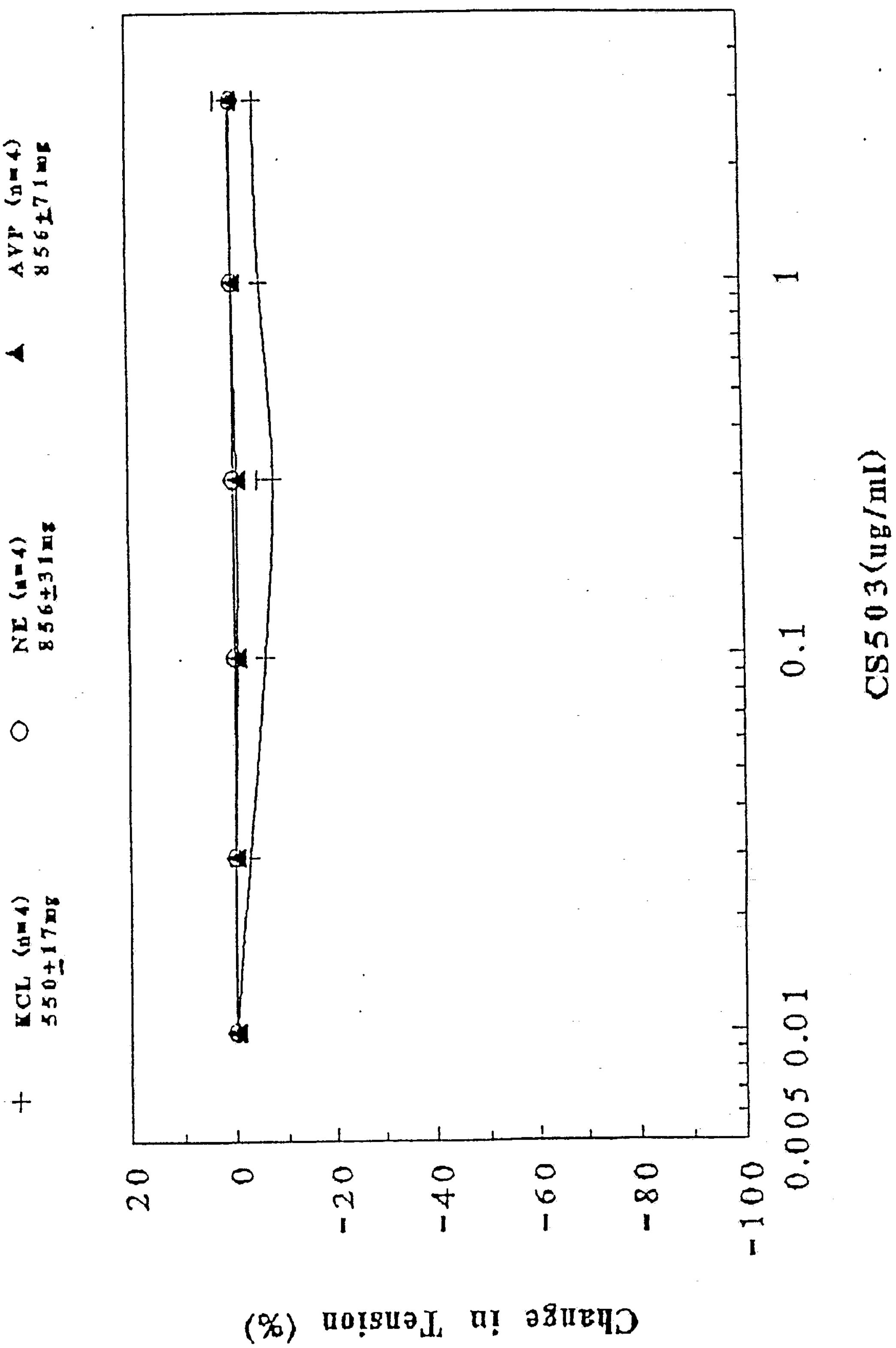


Fig 22

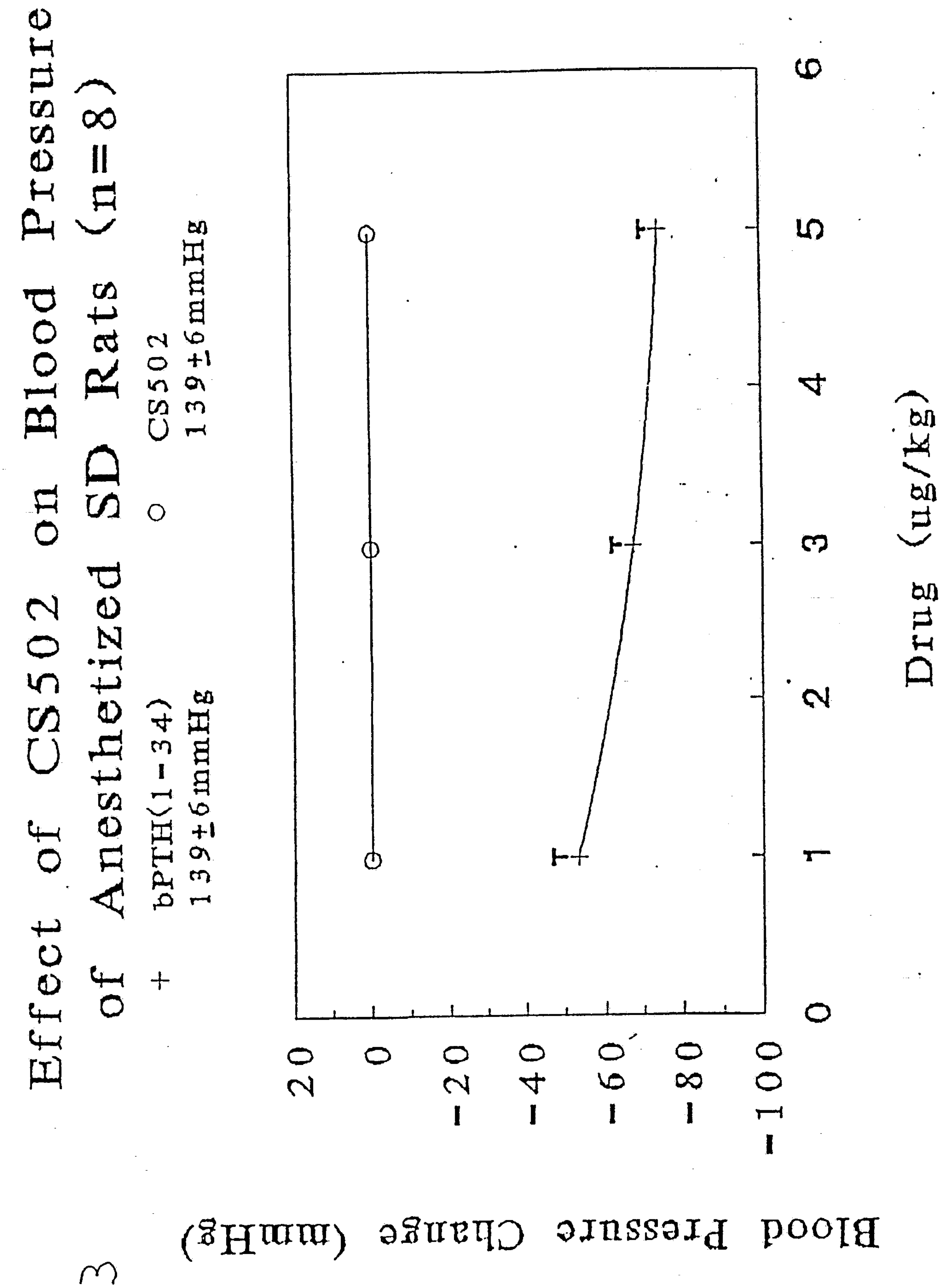

Effect of CS502 on Blood Pressure
of Anesthetized SD Rats (n=8)
+ bPTH(1-34)
139±6mmHg
○ CS502
139±6mmHg
20
0
-20
-40
-60
-80
-100
0
1
2
3
4
5
6
Drug (ug/kg)
Blood Pressure Change (mmHg)

Fig-23

Dose related relaxation curves produced by drug CS502 on SD rat tail artery helical strips precontracted with KCL (60 mM), NE ($10^{-[illegible]}$M) or AVP ($10^{-[illegible]}$M)

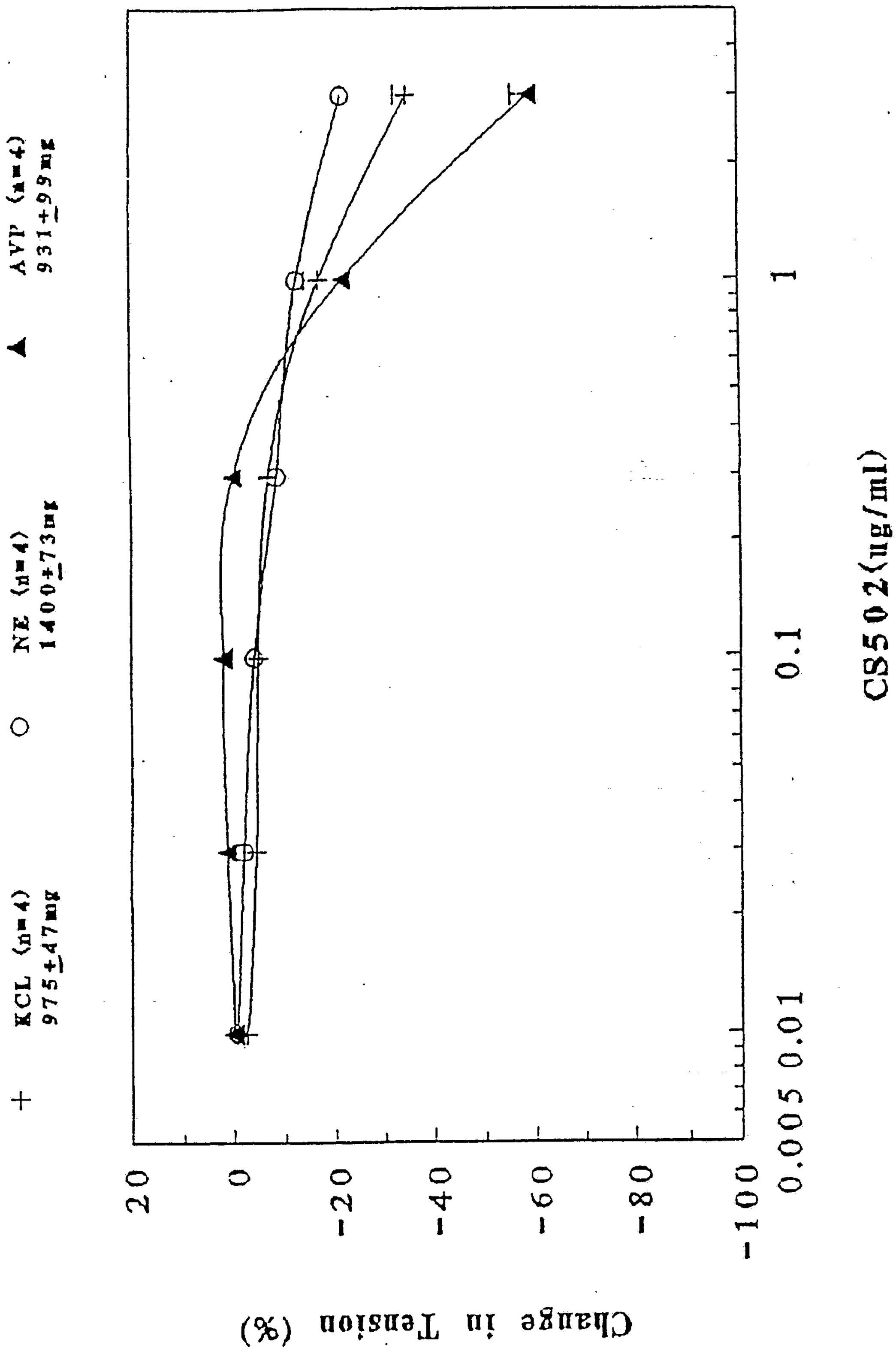


Fig. 24

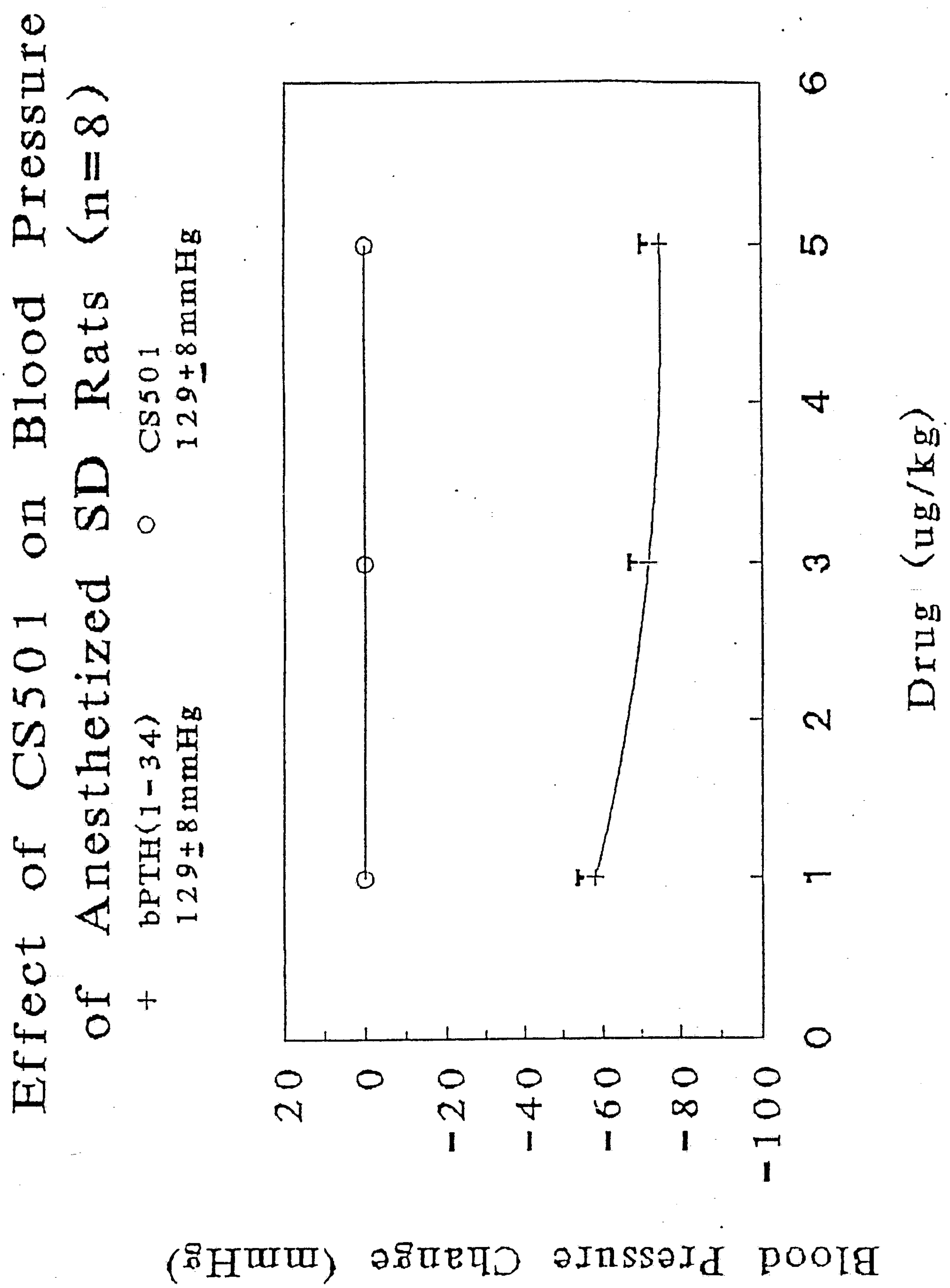

Effect of CS501 on Blood Pressure of Anesthetized SD Rats (n=8)
+ bPTH(1-34) 129±8mmHg
○ CS501 129±8mmHg
Blood Pressure Change (mmHg)
20
0
-20
-40
-60
-80
-100
0
1
2
3
4
5
6
Drug (ug/kg)

Fig 25

Dose related relaxation curves produced by drug CS501 on SD rat tail artery helical strips precontracted with KCL (60mM), NE (10^{-6}M) or AVP (10^{-8}M)

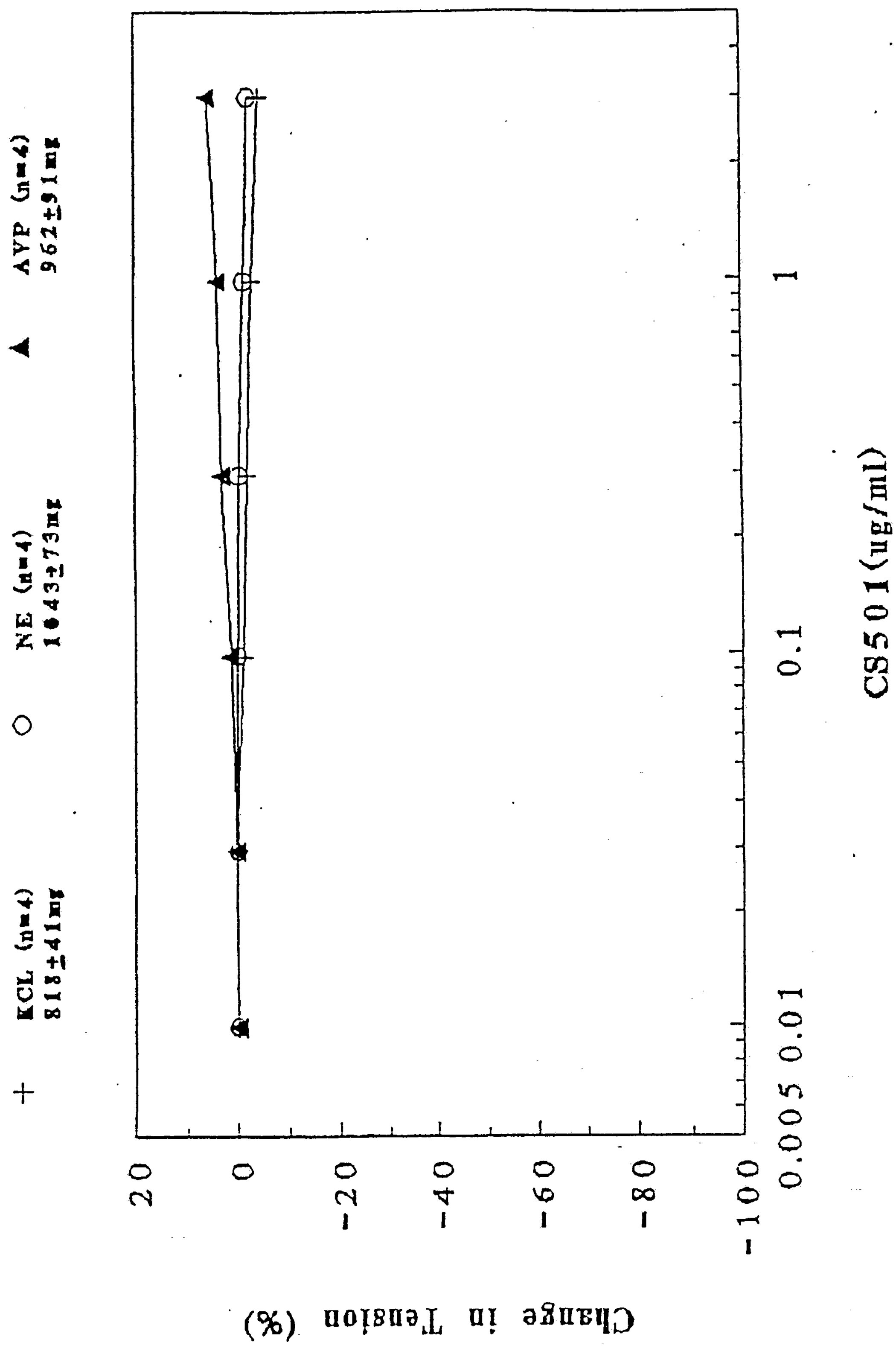


Fig 26

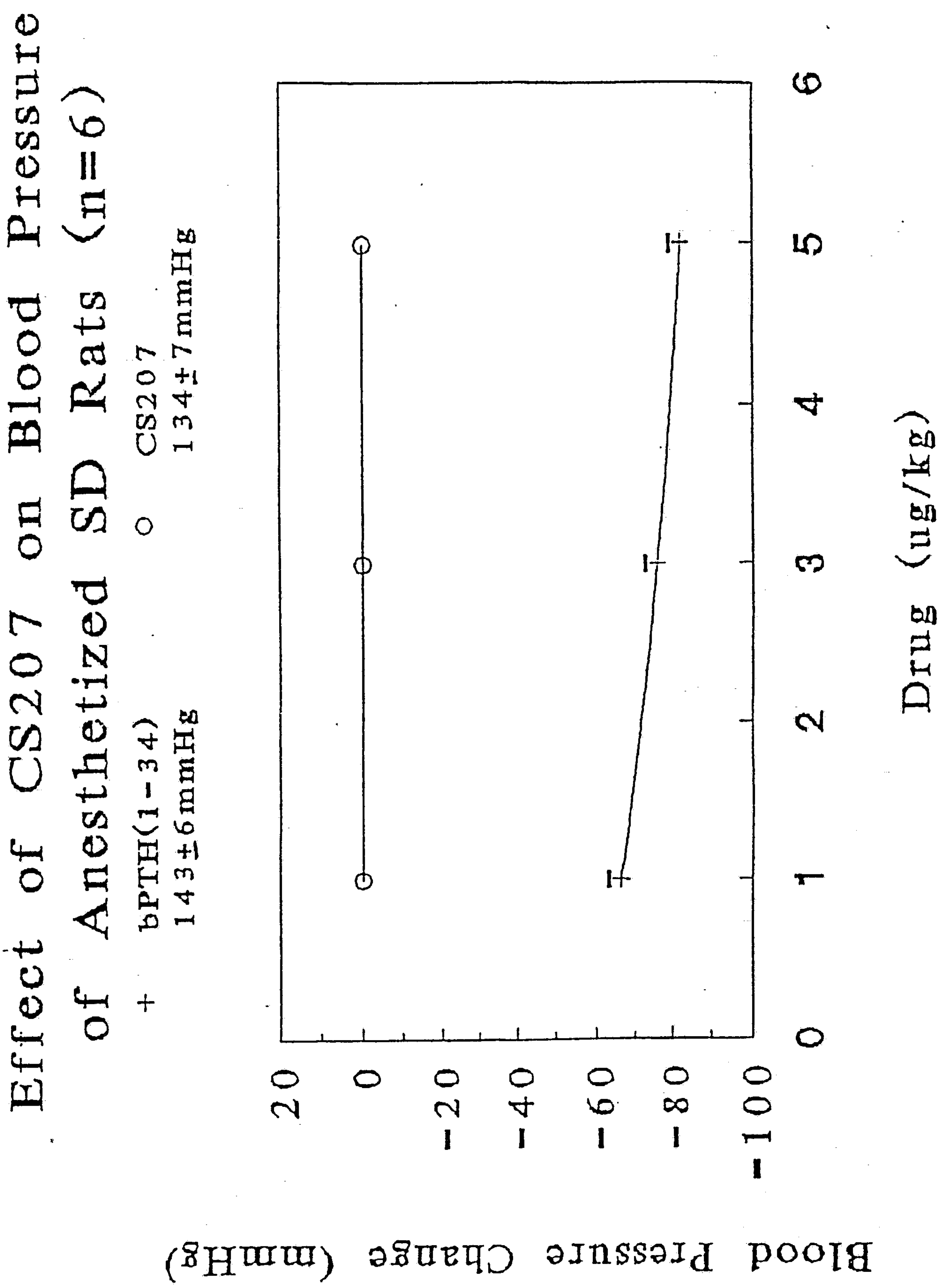

Effect of CS207 on Blood Pressure of Anesthetized SD Rats (n=6)
+ bPTH(1-34) 143±6mmHg
○ CS207 134±7mmHg
Blood Pressure Change (mmHg)
20
0
-20
-40
-60
-80
-100
0
1
2
3
4
5
6
Drug (ug/kg)

Fig. 27

Fig 28

Dose related relaxation curves procuced by drug CS207 on SD rat tail artery helical strips precontracted with KCL (60mM), NE (10^{-6}M) or AVP (10^{-6}M)

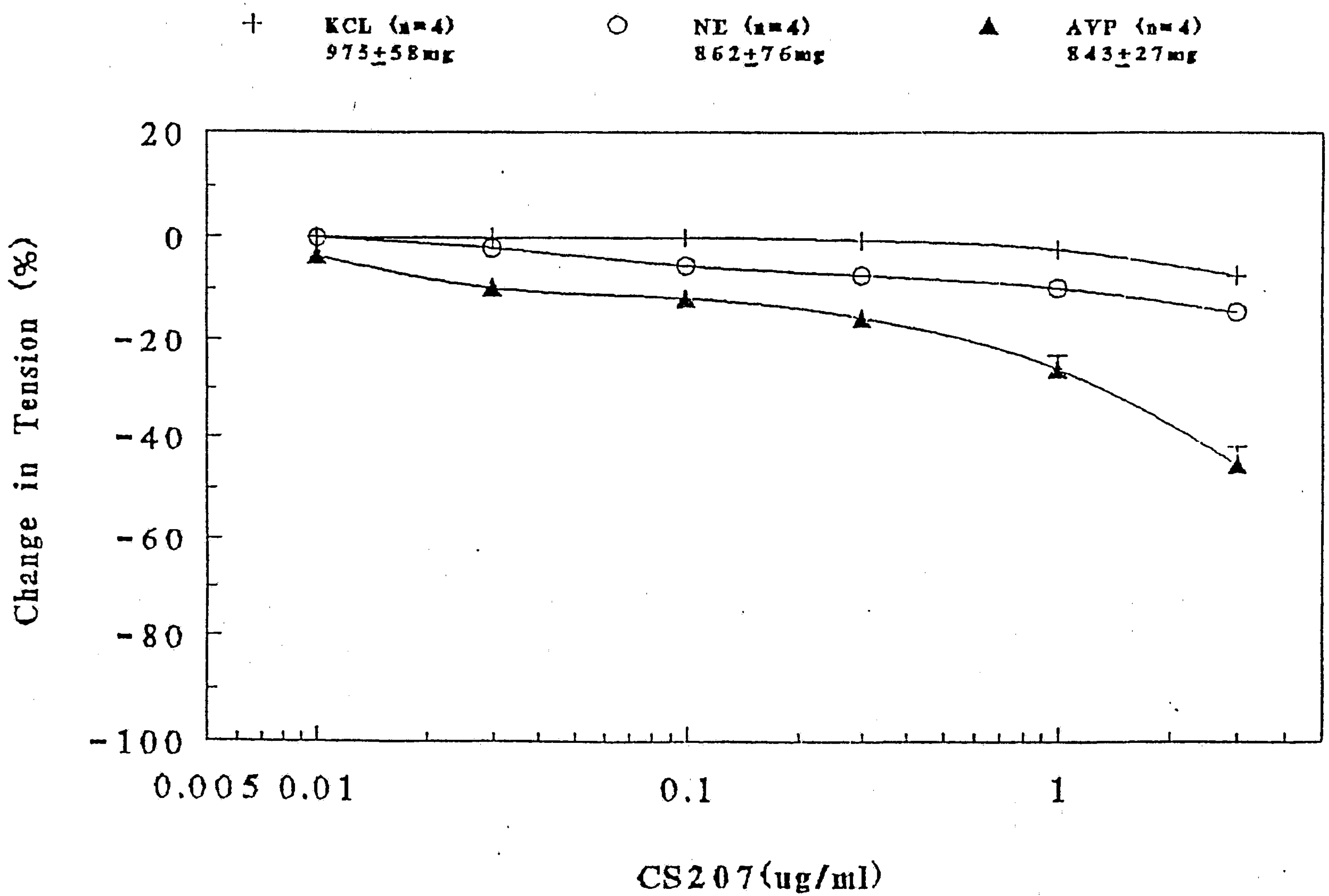
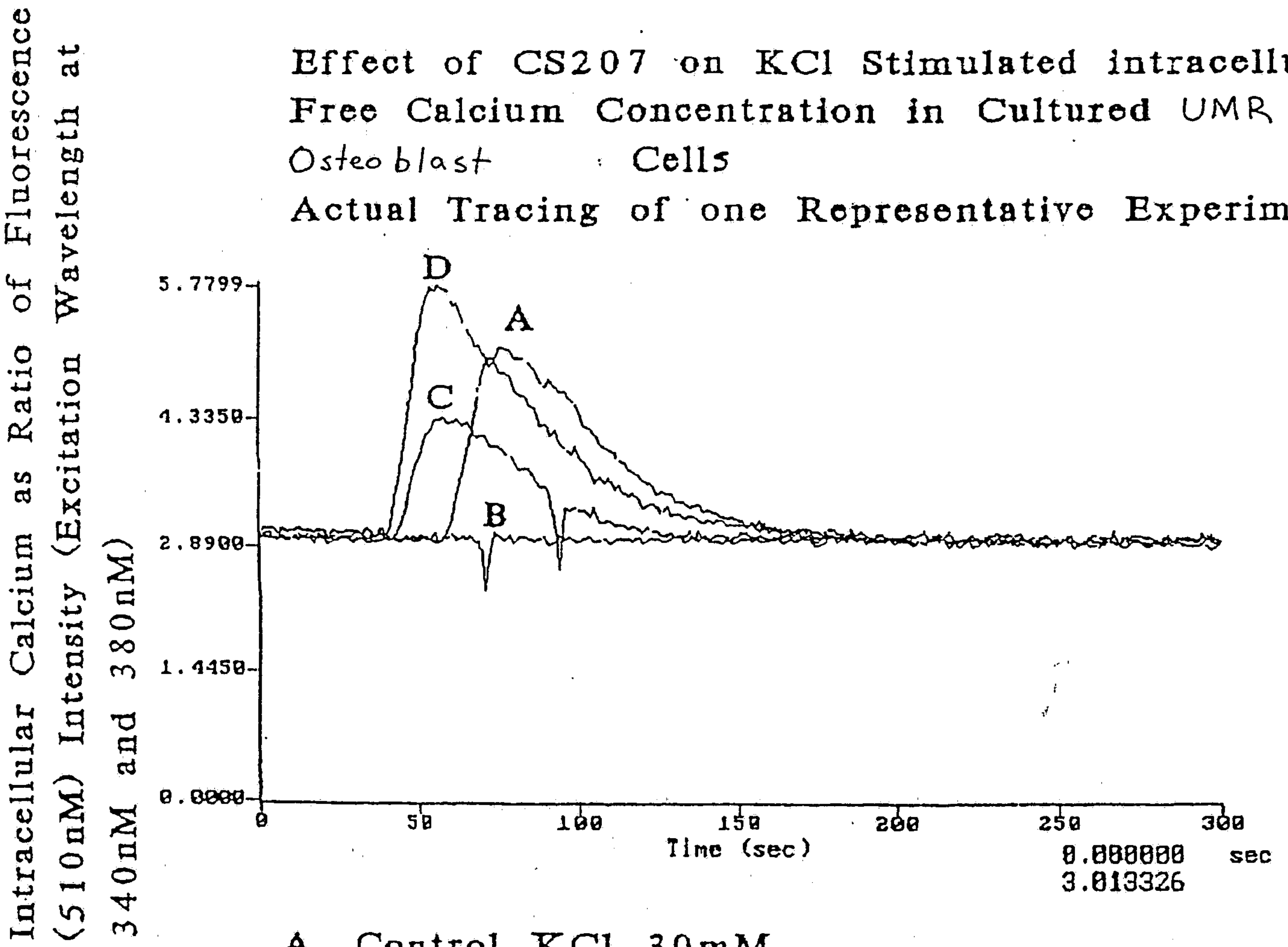


Fig 29

Effect of CS207 on KCl Stimulated intracellular Free Calcium Concentration in Cultured UMR Osteoblast Cells

Actual Tracing of one Representative Experiment



A. Control KCl 30mM
B. CS207 2ug/ml
C. KCl 30mM After CS207 10 Min
D. KCl 30mM After Wash

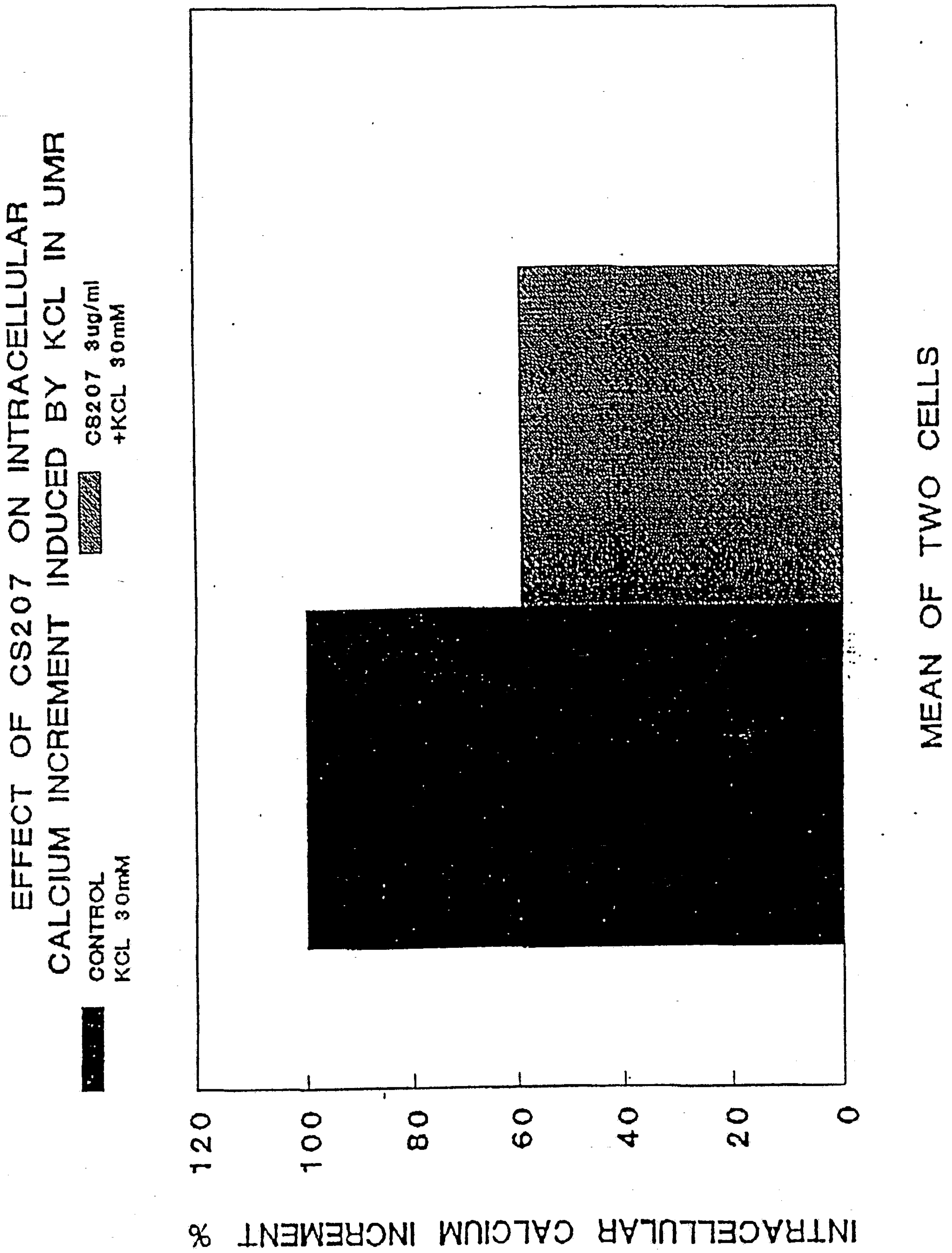

EFFECT OF CS207 ON INTRACELLULAR
CALCIUM INCREMENT INDUCED BY KCL IN UMR
CONTROL
KCL 30mM
CS207 3ug/ml
+KCL 30mM
INTRACELLULAR CALCIUM INCREMENT %
120
100
80
60
40
20
0
MEAN OF TWO CELLS

Fig 30

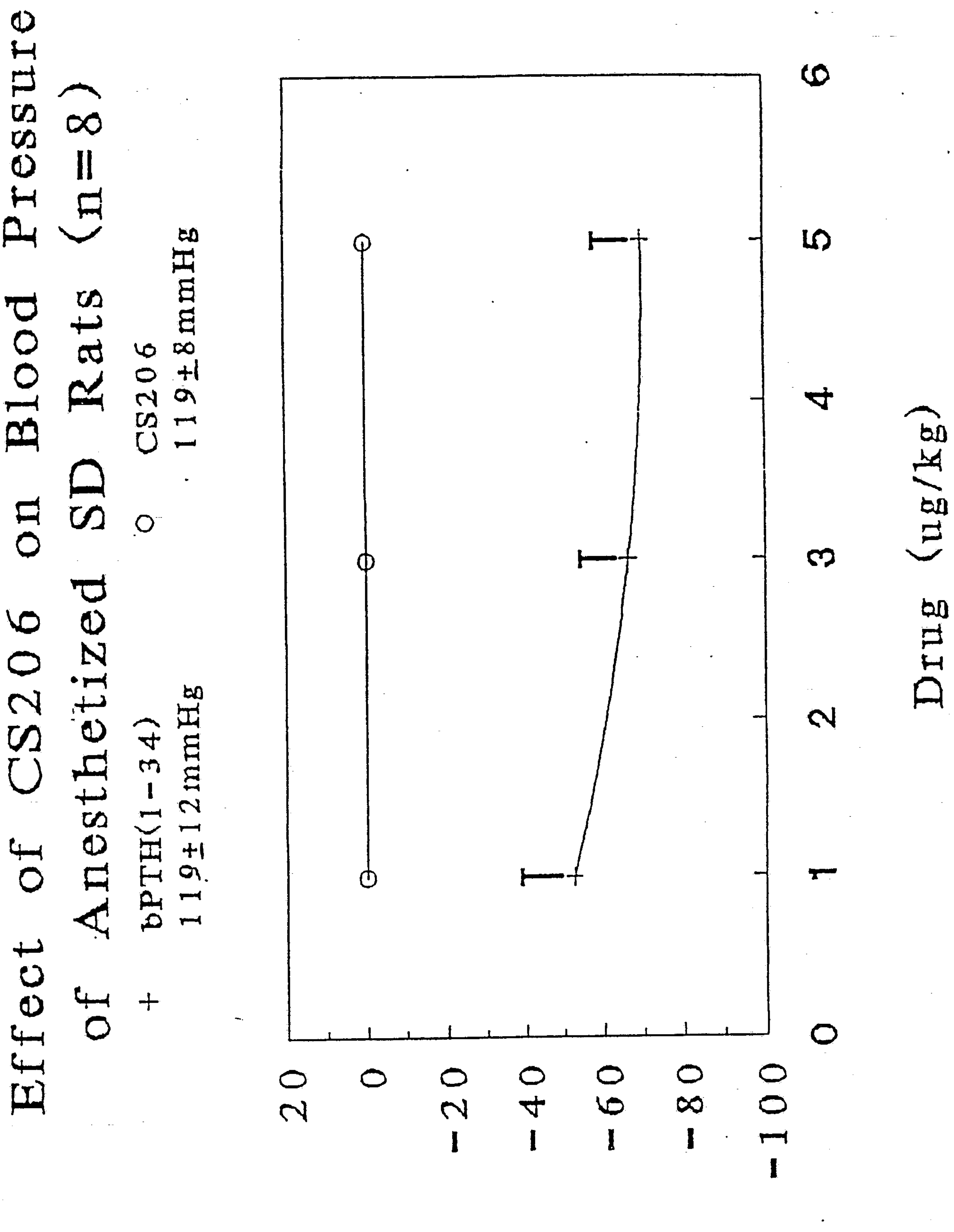

Effect of CS206 on Blood Pressure
of Anesthetized SD Rats (n=8)
+ bPTH(1-34)
119±12mmHg
○ CS206
119±8mmHg
20
0
-20
-40
-60
-80
-100
0
1
2
3
4
5
6
Drug (ug/kg)
Blood Pressure Change (mmHg)

Fig. 31

Fig.32

Dose related relaxation curves produced by drug CS206 on SD rat tail artery helical strips precontracted with KCL (60mM), NE (10^{-6}M) or AVP (10^{-8}M)

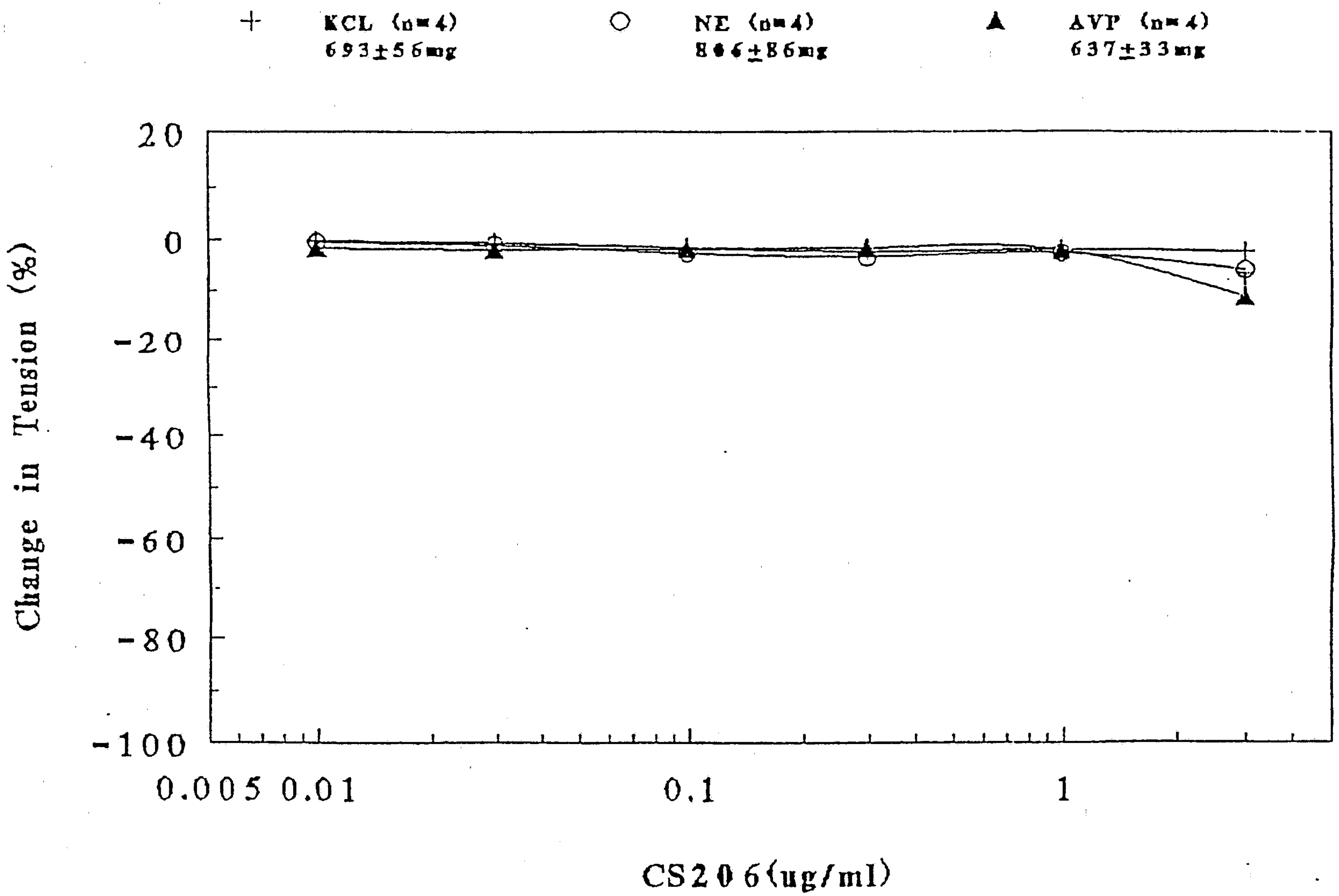
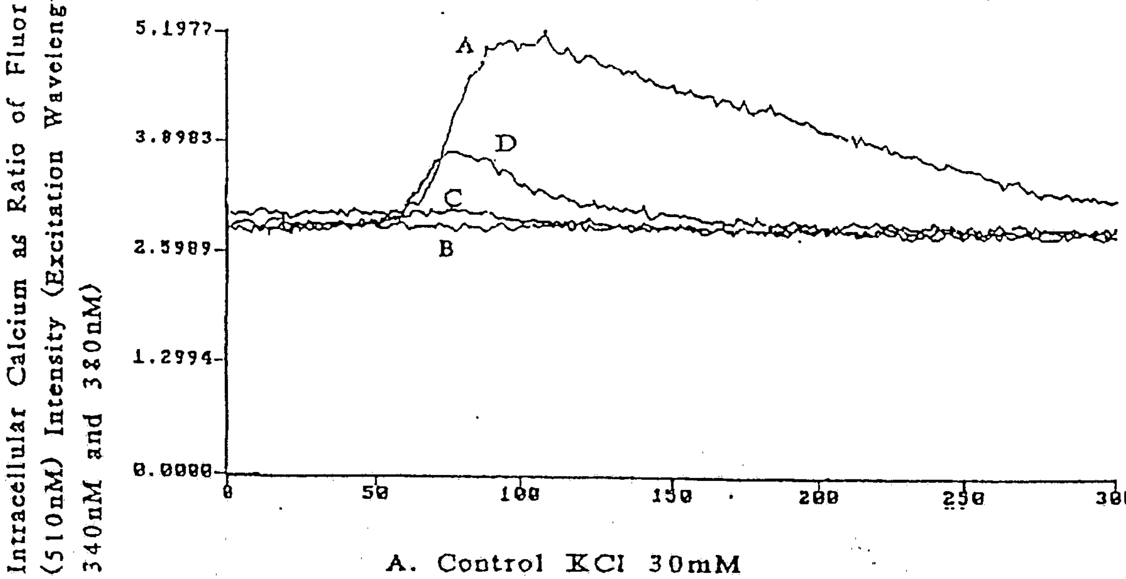


Fig. 33

Effect of CS206 on KCl Stimulated Intracellular Free Calcium Concentration in Cultured UMR Osteoblast Cells

Actual Tracing of one Representative Experiment



A. Control KCl 30mM
B. CS206 2ug/ml
C. KCl 30mM After CS206 10 Min
D. KCl 30mM After Wash

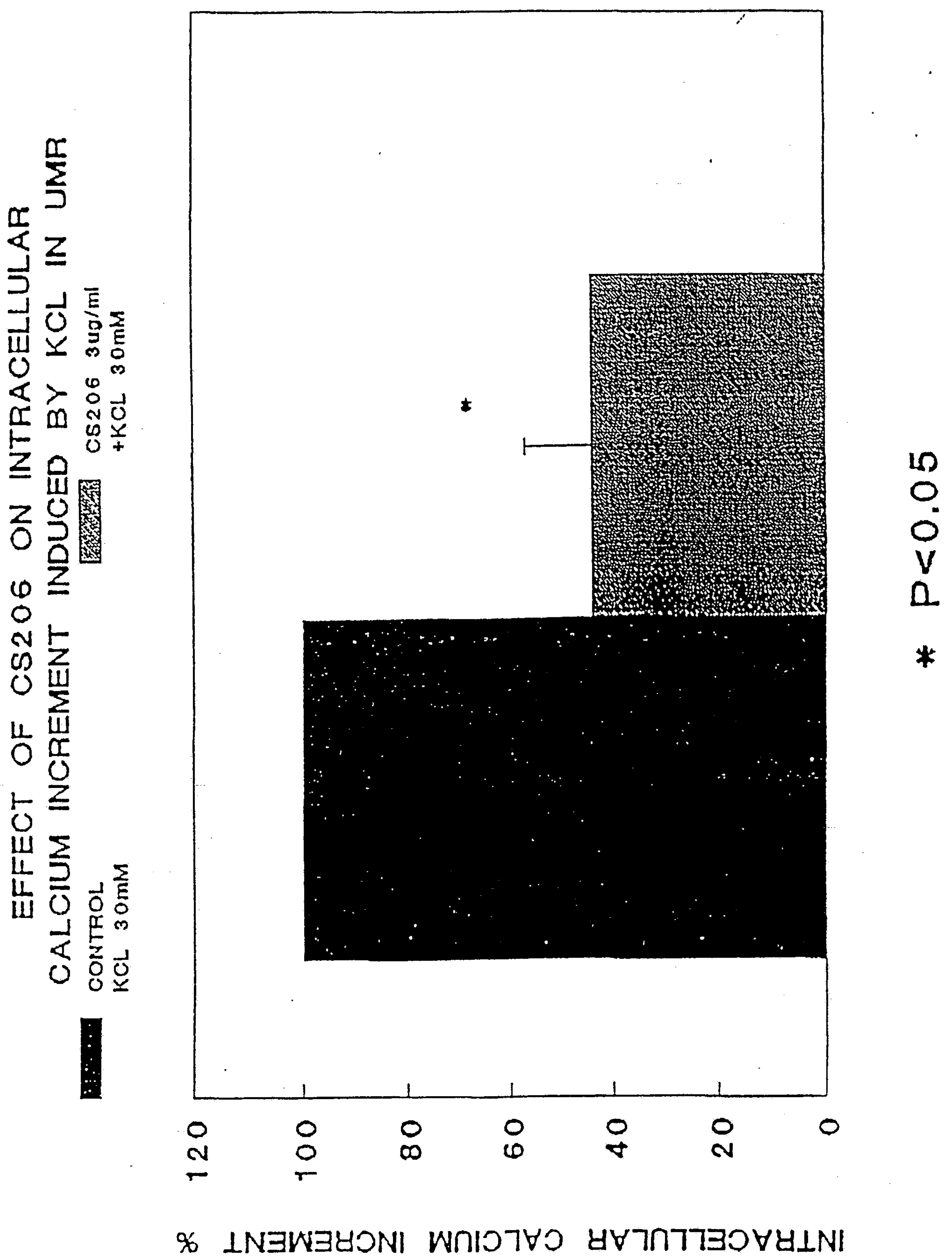

EFFECT OF CS206 ON INTRACELLULAR CALCIUM INCREMENT INDUCED BY KCL IN UMR
CONTROL KCL 30mM
CS206 3ug/ml +KCL 30mM
INTRACELLULAR CALCIUM INCREMENT %
120
100
80
60
40
20
0
*
* P<0.05

Fig. 34

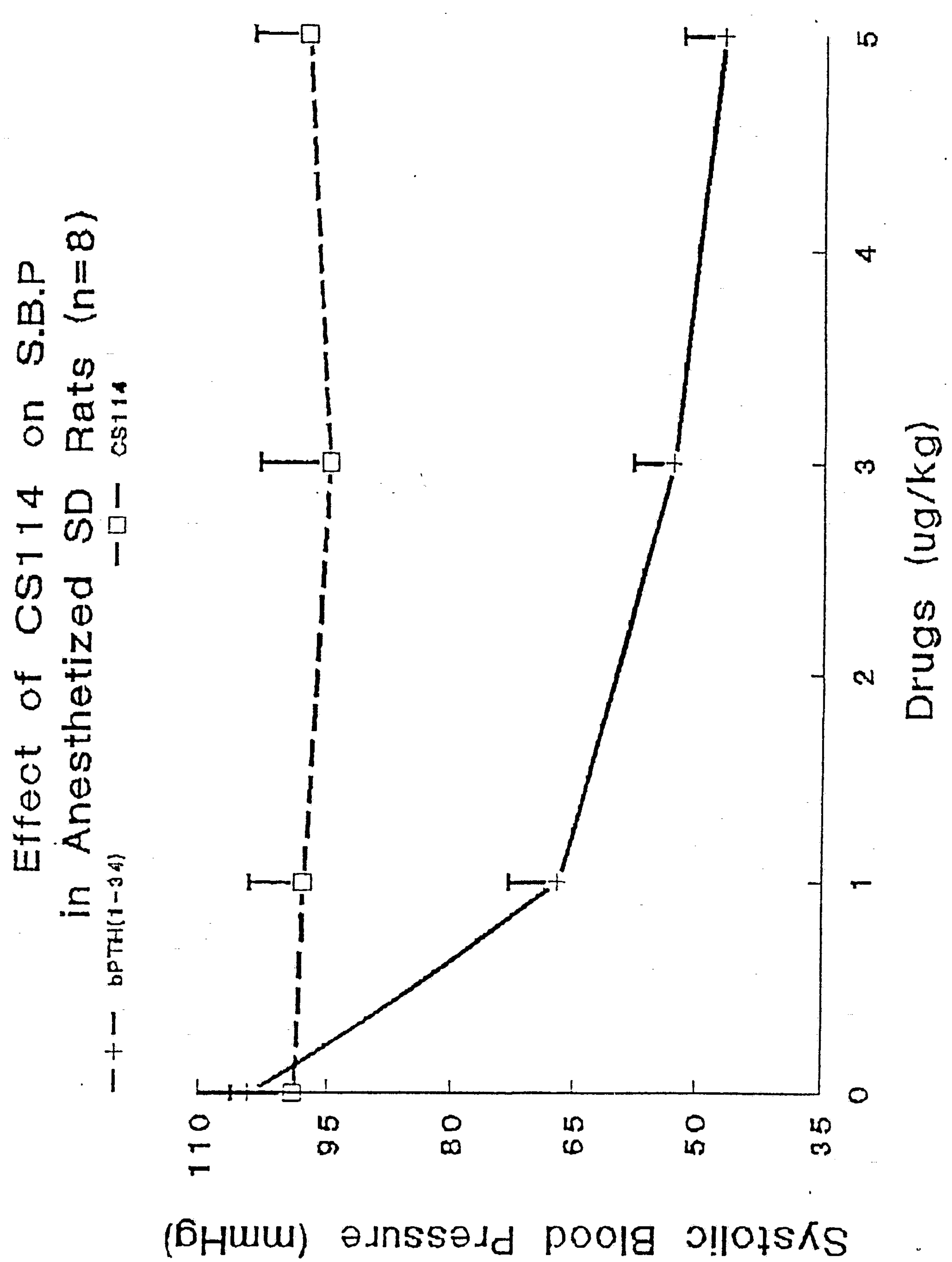

Effect of CS114 on S.B.P
in Anesthetized SD Rats (n=8)
bPTH(1-34)
CS114
Systolic Blood Pressure (mmHg)
110
95
80
65
50
35
0
1
2
3
4
5
Drugs (ug/kg)

Fig. 35

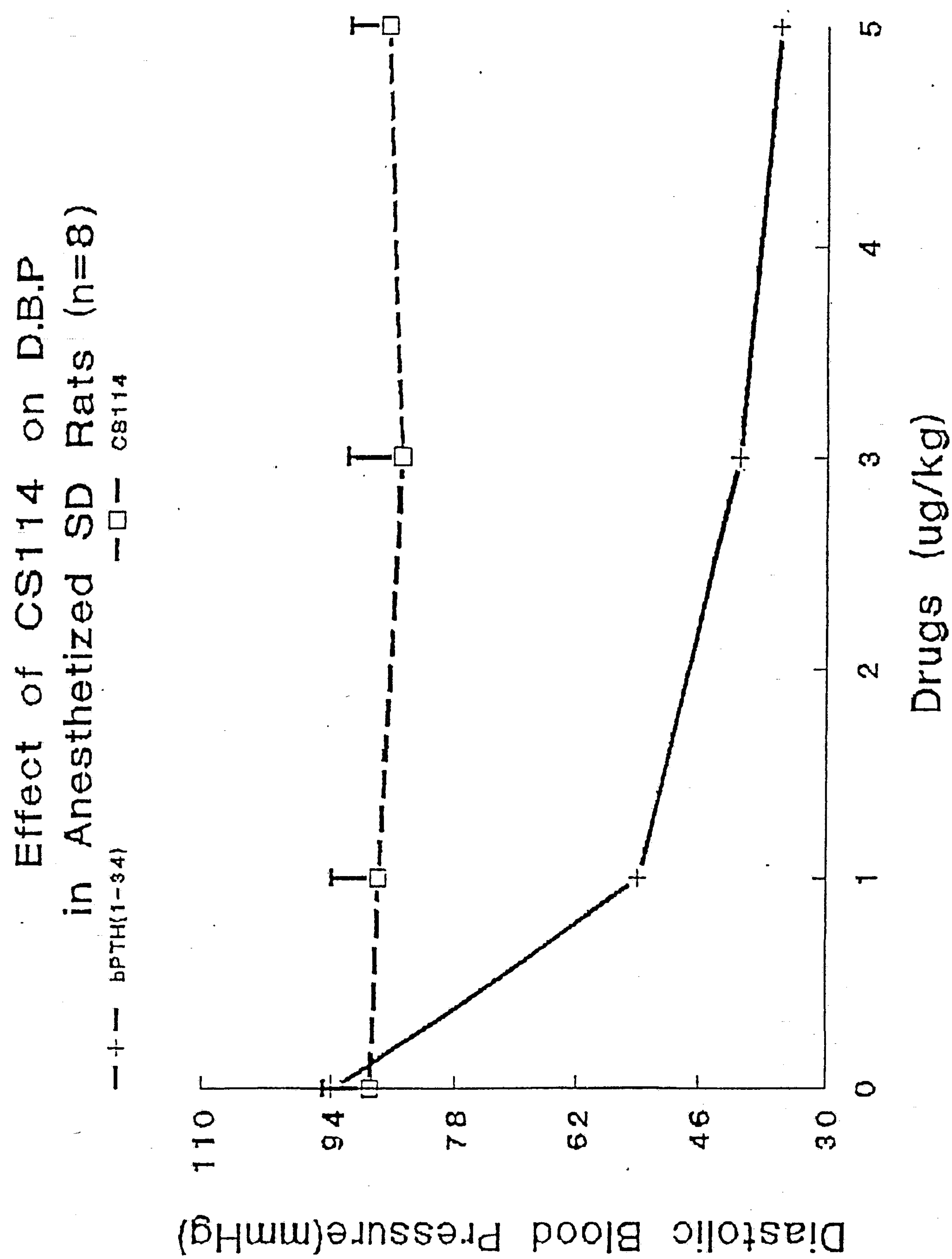

Effect of CS114 on D.B.P
in Anesthetized SD Rats (n=8)
bPTH(1-34)
CS114
Diastolic Blood Pressure(mmHg)
110
94
78
62
46
30
0
1
2
3
4
5
Drugs (ug/kg)

Fig-36

Fig. 37 Dose related relaxation curves produced by drug CS114 on SD rat tail artery helical strips precontracted with NE (10^{-6}M), AVP (10^{-8}M) or KCl (60 mM)

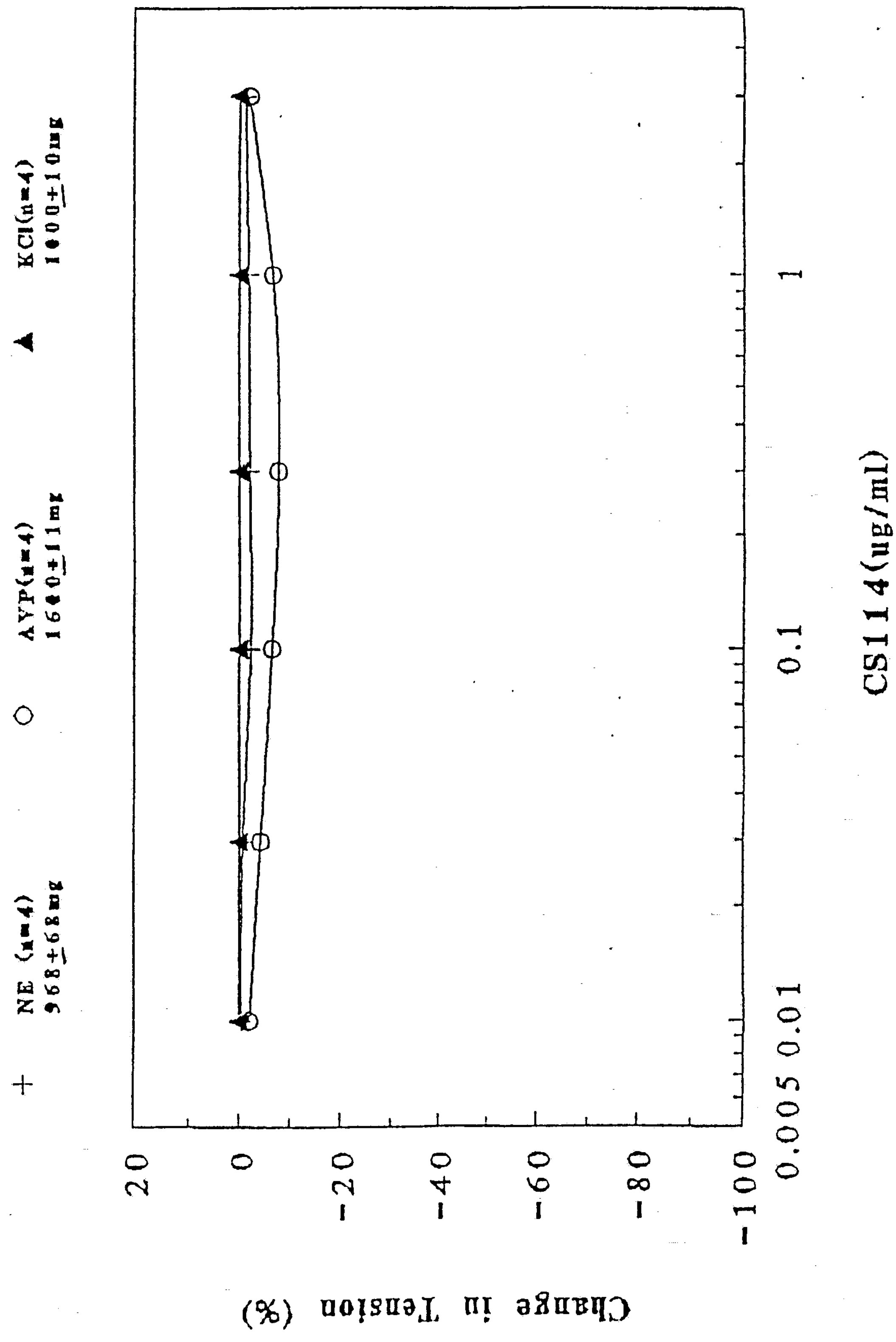
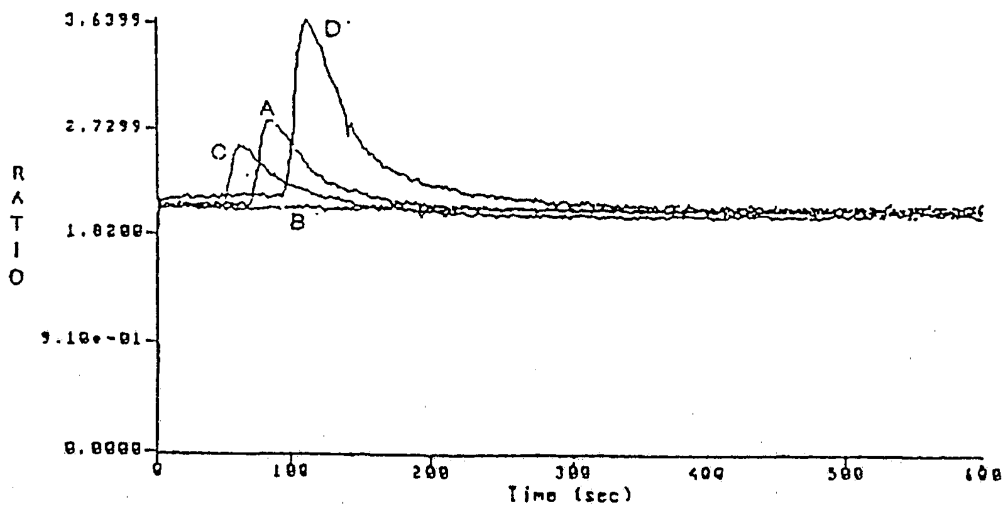


Fig 38

Effect of CS114 on KCl Stimulated intracellular Free Calcium Concentration in Cultured UMR Osteoblast Cells

Actual Tracing of one Representative Experiment



Intracellular Calcium as Ratio of Fluorescence (510nM) Intensity (Excitation Wavelength at 340nM and 380nM)

A. Control KCl 15mM
B. CS114 2.5X10-6M
C. KCl 15mM After CS114 10 Min
D. KCl 15mM After Wash

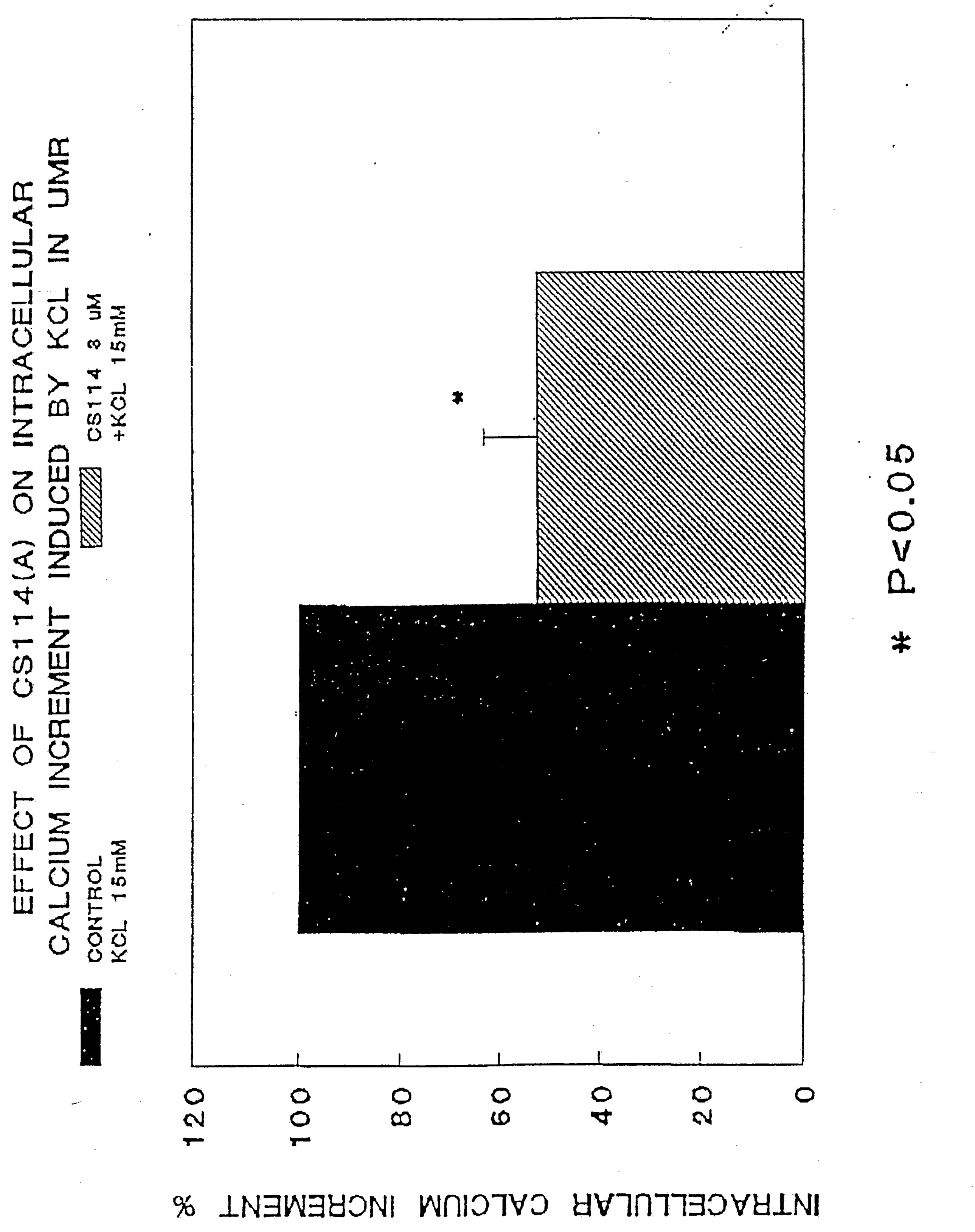

EFFECT OF CS114(A) ON INTRACELLULAR
CALCIUM INCREMENT INDUCED BY KCL IN UMR
CONTROL
KCL 15mM
CS114 3 uM
+KCL 15mM
*
120
100
80
60
40
20
0
INTRACELLULAR CALCIUM INCREMENT %
* P<0.05

Fig. 39

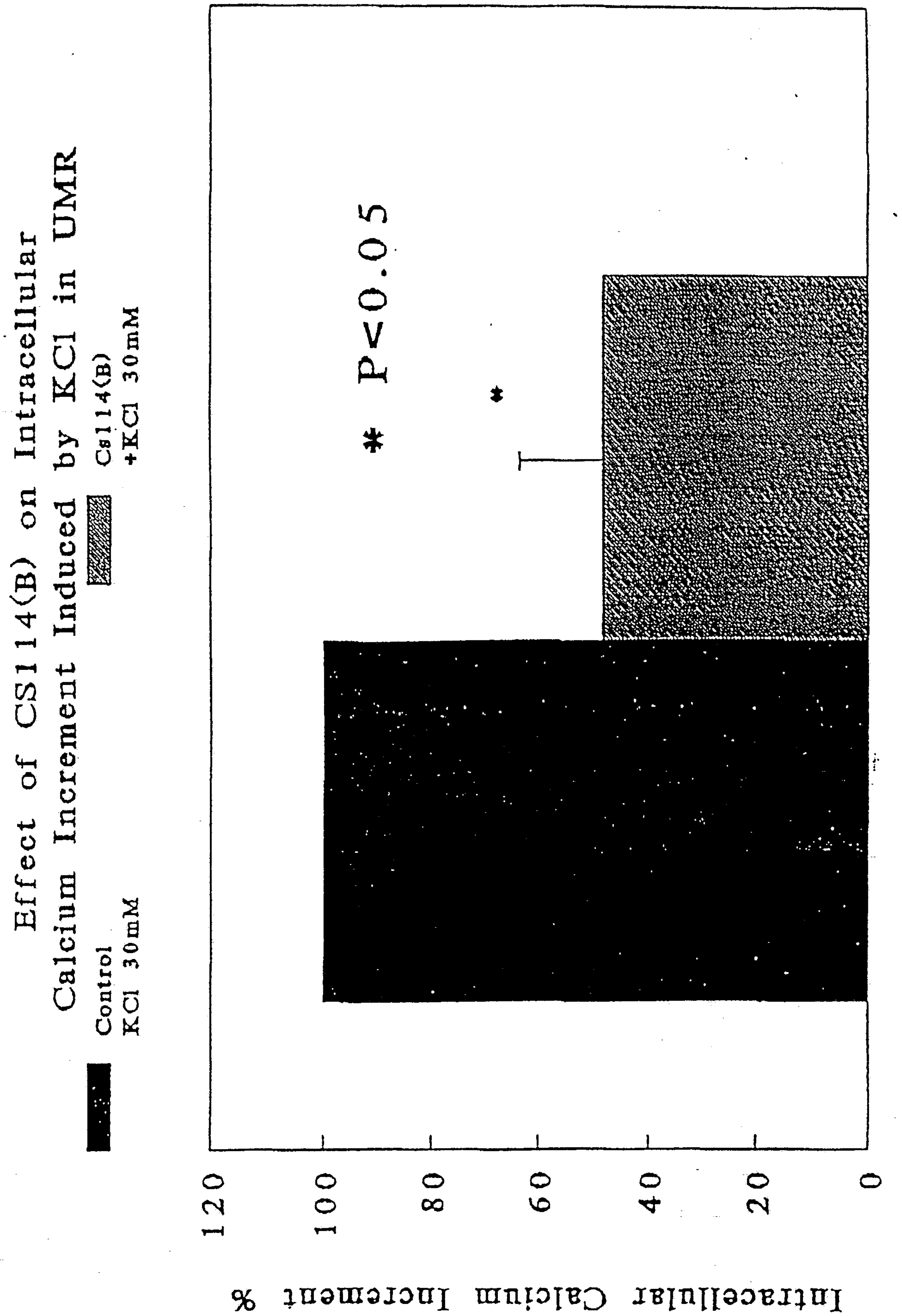

Effect of CS114(B) on Intracellular
Calcium Increment Induced by KCl in UMR
Control
KCl 30mM
Cs114(B)
+KCl 30mM
* P<0.05
*
120
100
80
60
40
20
0
Intracellular Calcium Increment %

Fig. 40

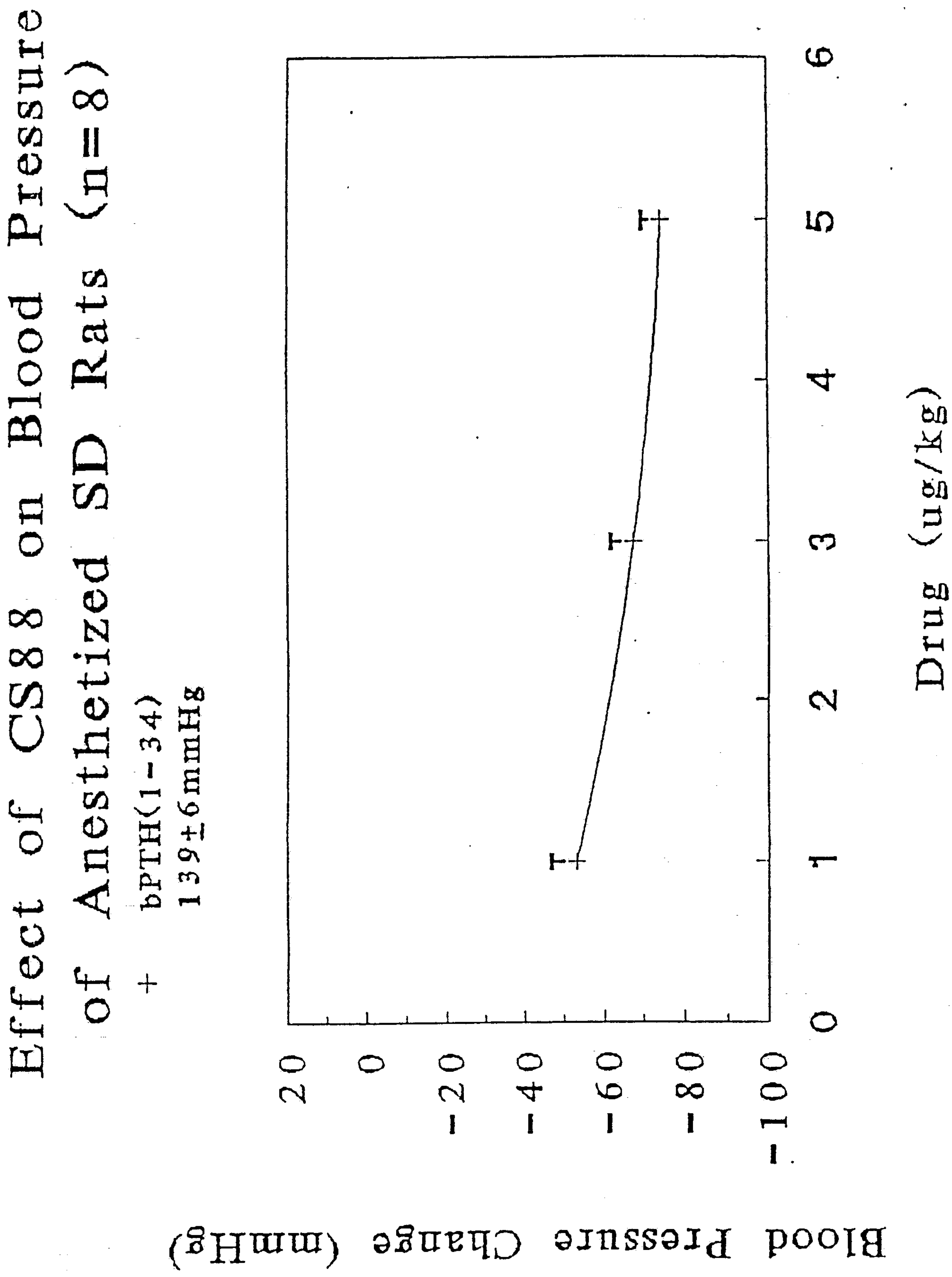

Effect of CS88 on Blood Pressure
of Anesthetized SD Rats (n=8)
+ bPTH(1-34)
139±6mmHg
Blood Pressure Change (mmHg)
20
0
-20
-40
-60
-80
-100
0
1
2
3
4
5
6
Drug (ug/kg)

Fig. 41

Fig 42

Dose related relaxation curves produced by drug bPTH on SD rat tail artery helical strips precontracted with KCL (60mM), NE ($3x10^{-4}$M) or AVP 10^{-8}M)

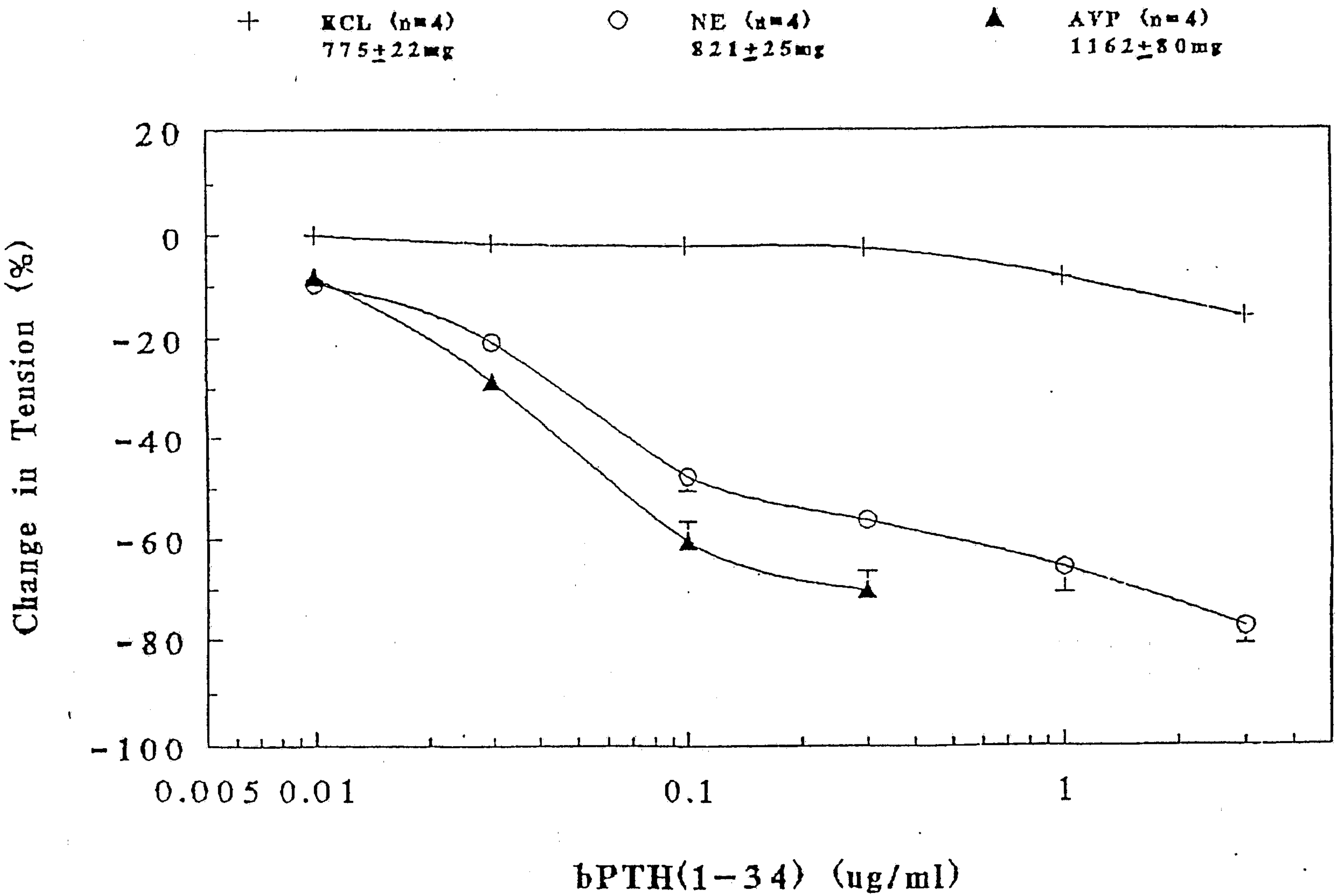
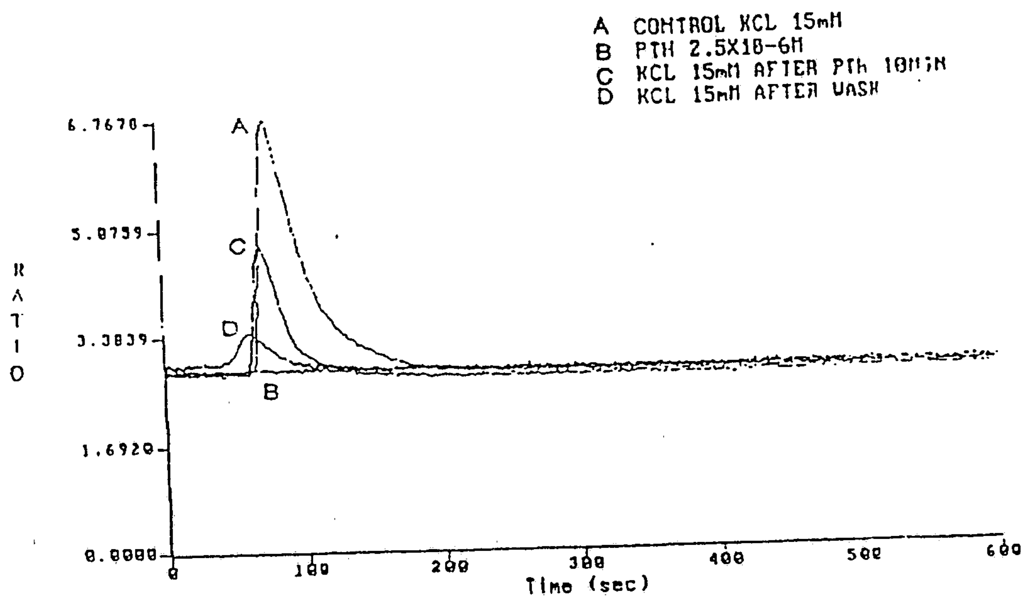


Fig. 43

Effect of CS88 on KCl Stimulated intracellular Free Calcium Concentration in Cultured UMR Osteoblast Cells

Actual Tracing of one Representative Experiment



Intracellular Calcium as Ratio of Fluorescence (510nM) Intensity (Excitation Wavelength at 340nM and 380nM)

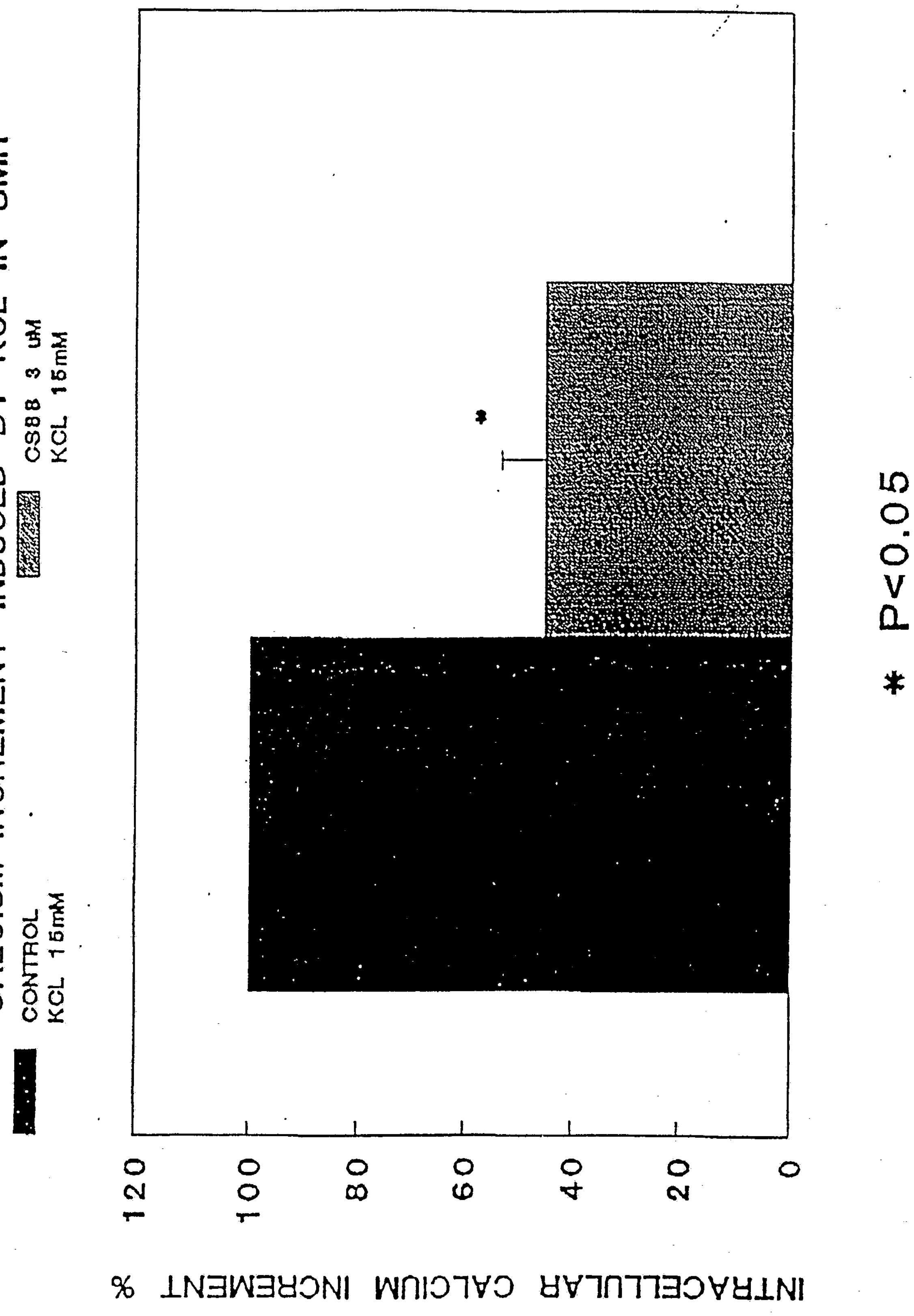

EFFECT OF CS88 ON INTRACELLULAR
CALCIUM INCREMENT INDUCED BY KCL IN UMR
CONTROL
KCL 15mM
CS88 3 uM
KCL 15mM
*
120
100
80
60
40
20
0
INTRACELLULAR CALCIUM INCREMENT %
* P<0.05

Fig. 44

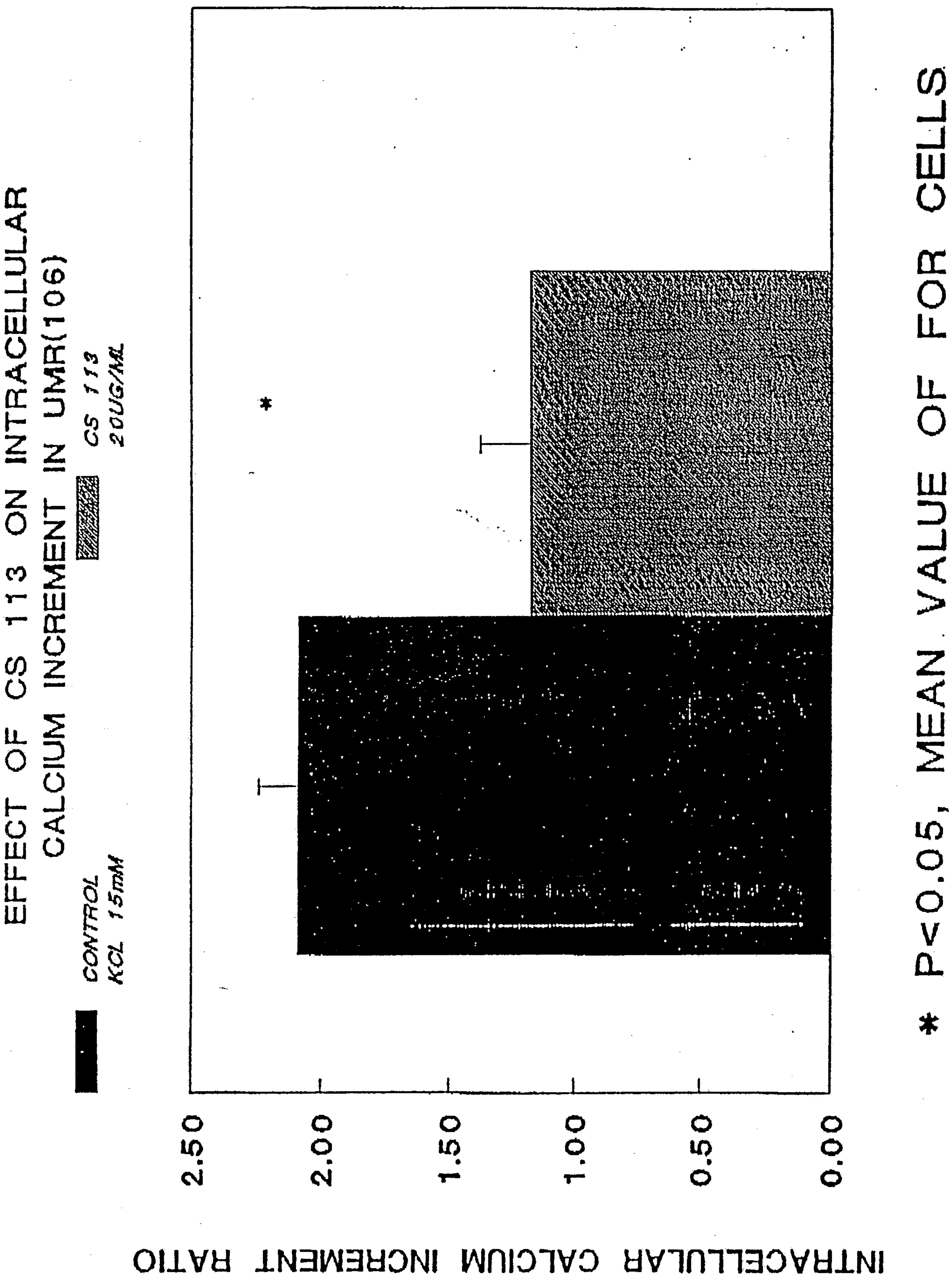
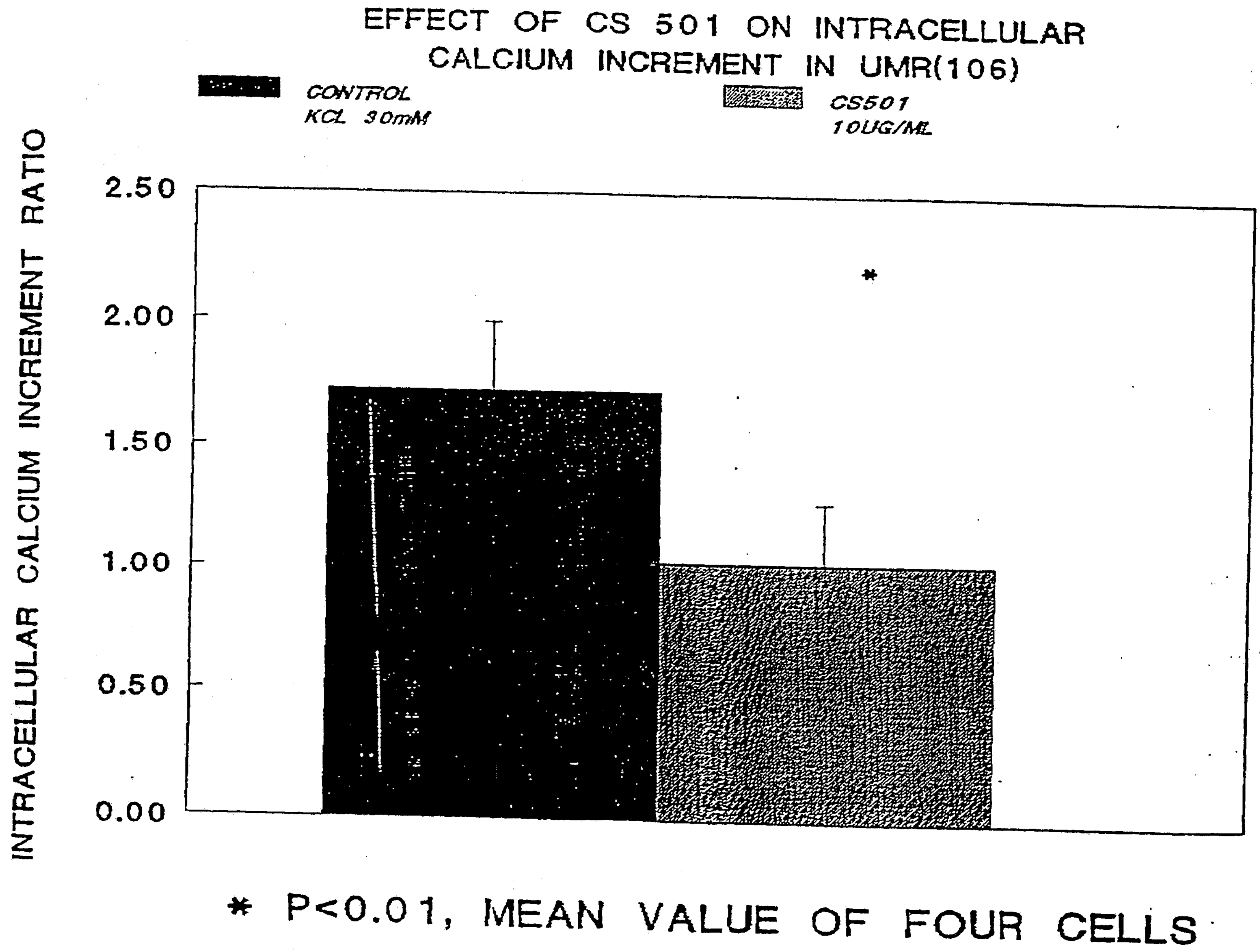

EFFECT OF CS 113 ON INTRACELLULAR
CALCIUM INCREMENT IN UMR(106)
CONTROL
KCL 15mM
CS 113
20UG/ML
*
2.50
2.00
1.50
1.00
0.50
0.00
INTRACELLULAR CALCIUM INCREMENT RATIO
* P<0.05, MEAN VALUE OF FOR CELLS

Fig. 45

Fig. 46


EFFECT OF CS 501 ON INTRACELLULAR
CALCIUM INCREMENT IN UMR(106)
CONTROL
KCL 30mM
CS501
10UG/ML
INTRACELLULAR CALCIUM INCREMENT RATIO
2.50
2.00
1.50
1.00
0.50
0.00
*
* P<0.01, MEAN VALUE OF FOUR CELLS

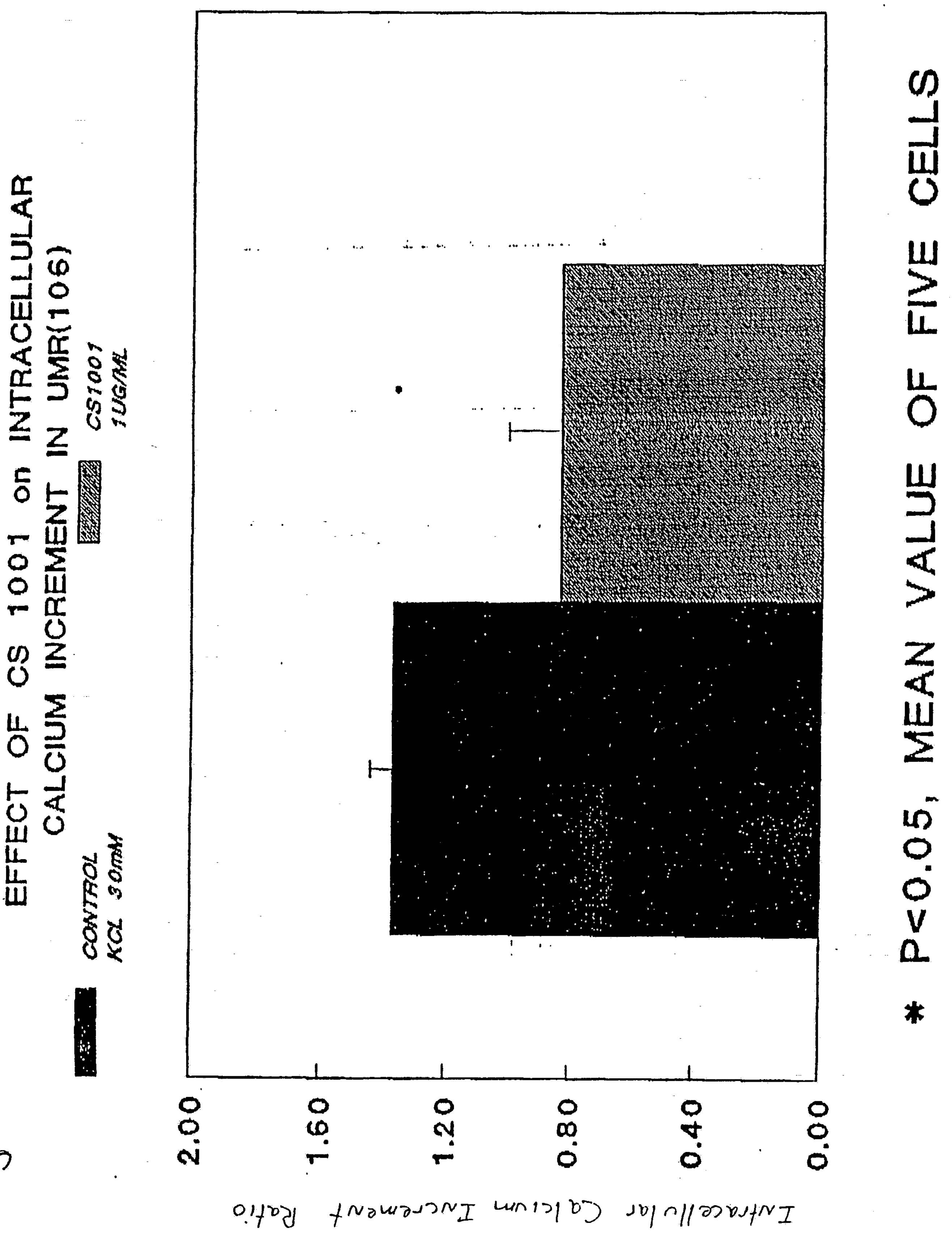
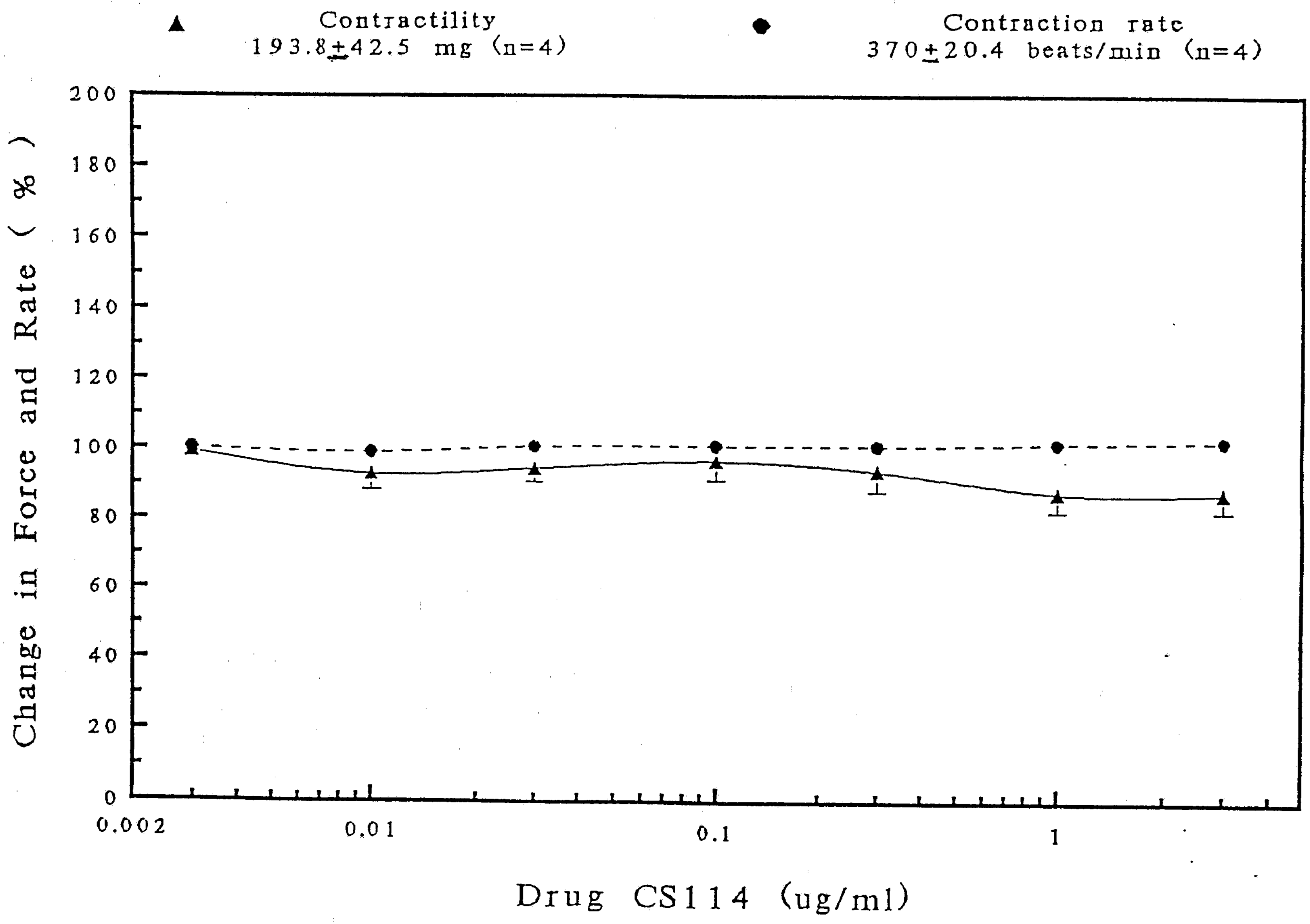

EFFECT OF CS 1001 on INTRACELLULAR
CALCIUM INCREMENT IN UMR(106)
CONTROL
KCL 30mM
CS1001
1UG/ML
2.00
1.60
1.20
0.80
0.40
0.00
Intracellular Calcium Increment Ratio
* P<0.05, MEAN VALUE OF FIVE CELLS

Fig 47

Fig 48

Dose related response curves of right atrial (SD rat) contractility and contraction rate by drug CS114



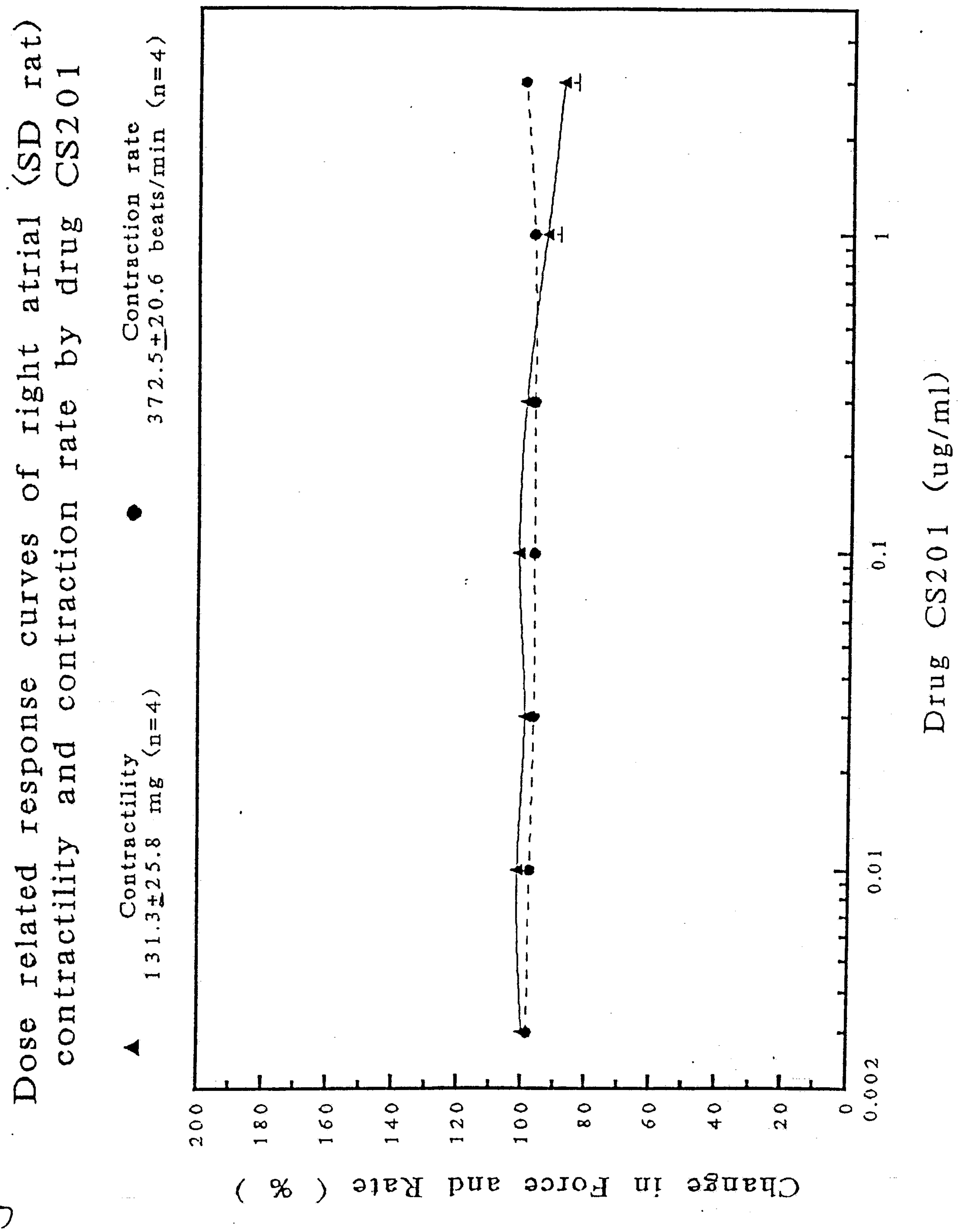

Dose related response curves of right atrial (SD rat) contractility and contraction rate by drug CS201
▲ Contractility 131.3±25.8 mg (n=4)
● Contraction rate 372.5±20.6 beats/min (n=4)
Change in Force and Rate (%)
200
180
160
140
120
100
80
60
40
20
0
0.002
0.01
0.1
1
Drug CS201 (ug/ml)

Fig 49

Dose related response curves of right atrial (SD rat) contractility and contraction rate by drug CS205

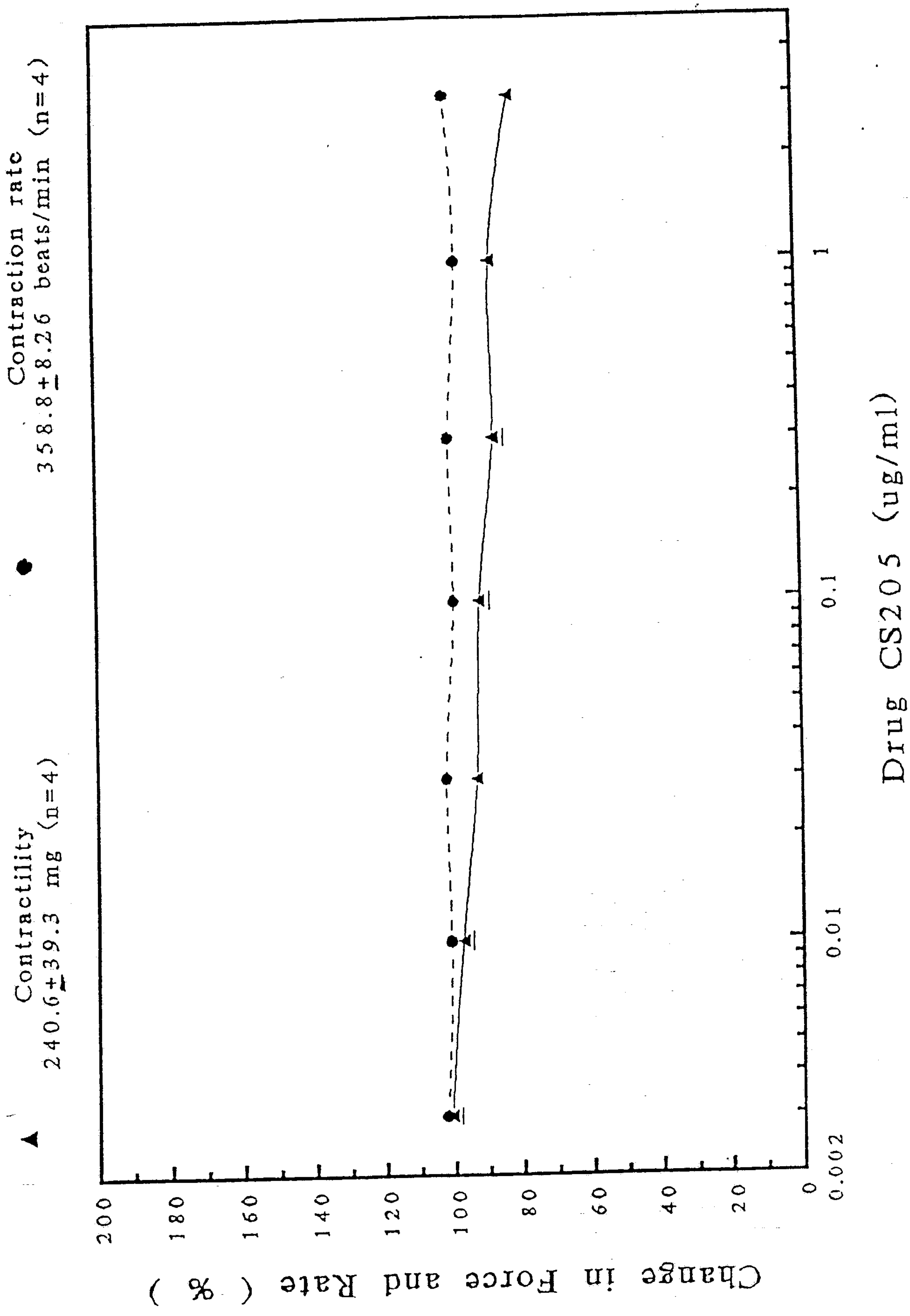
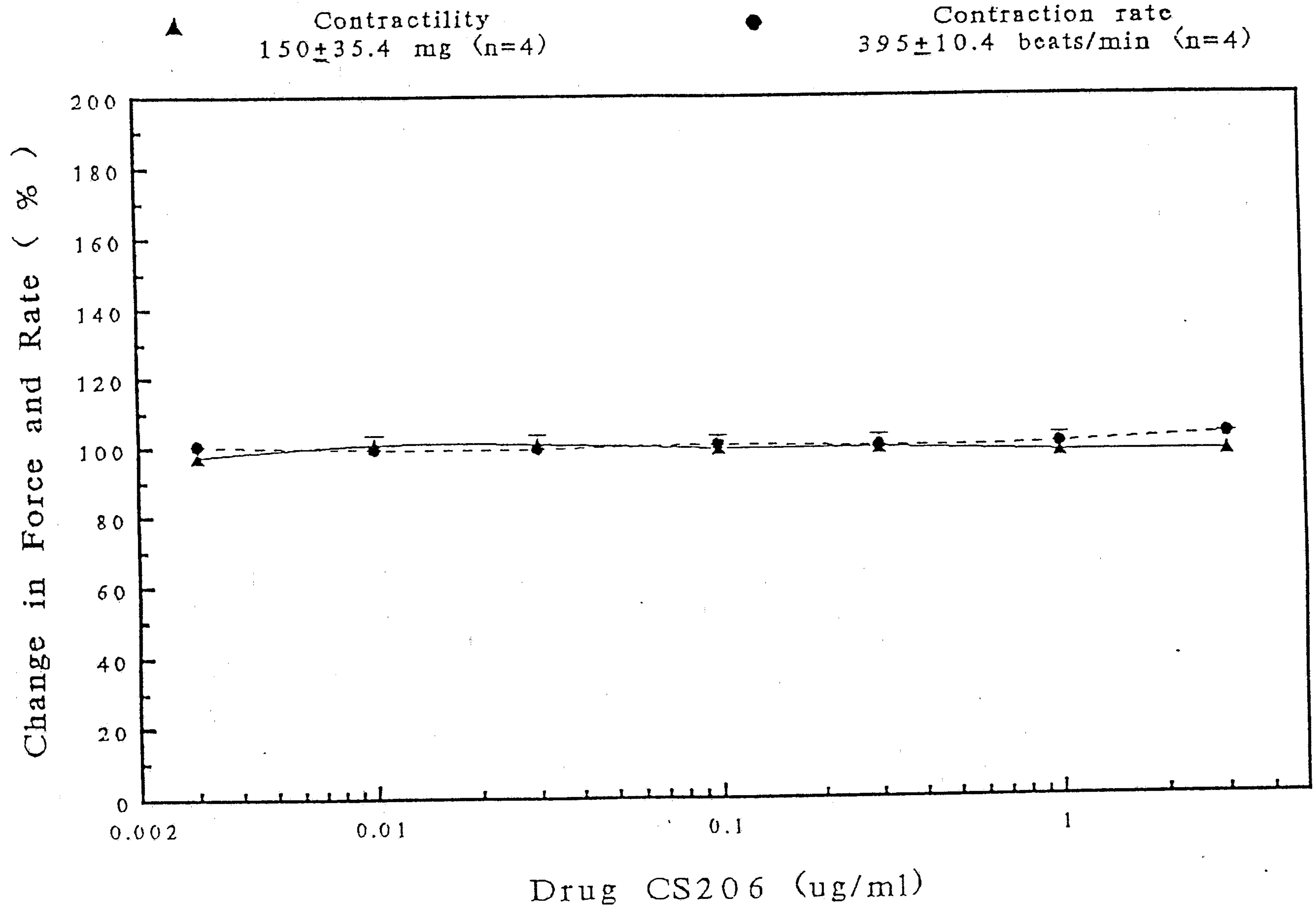
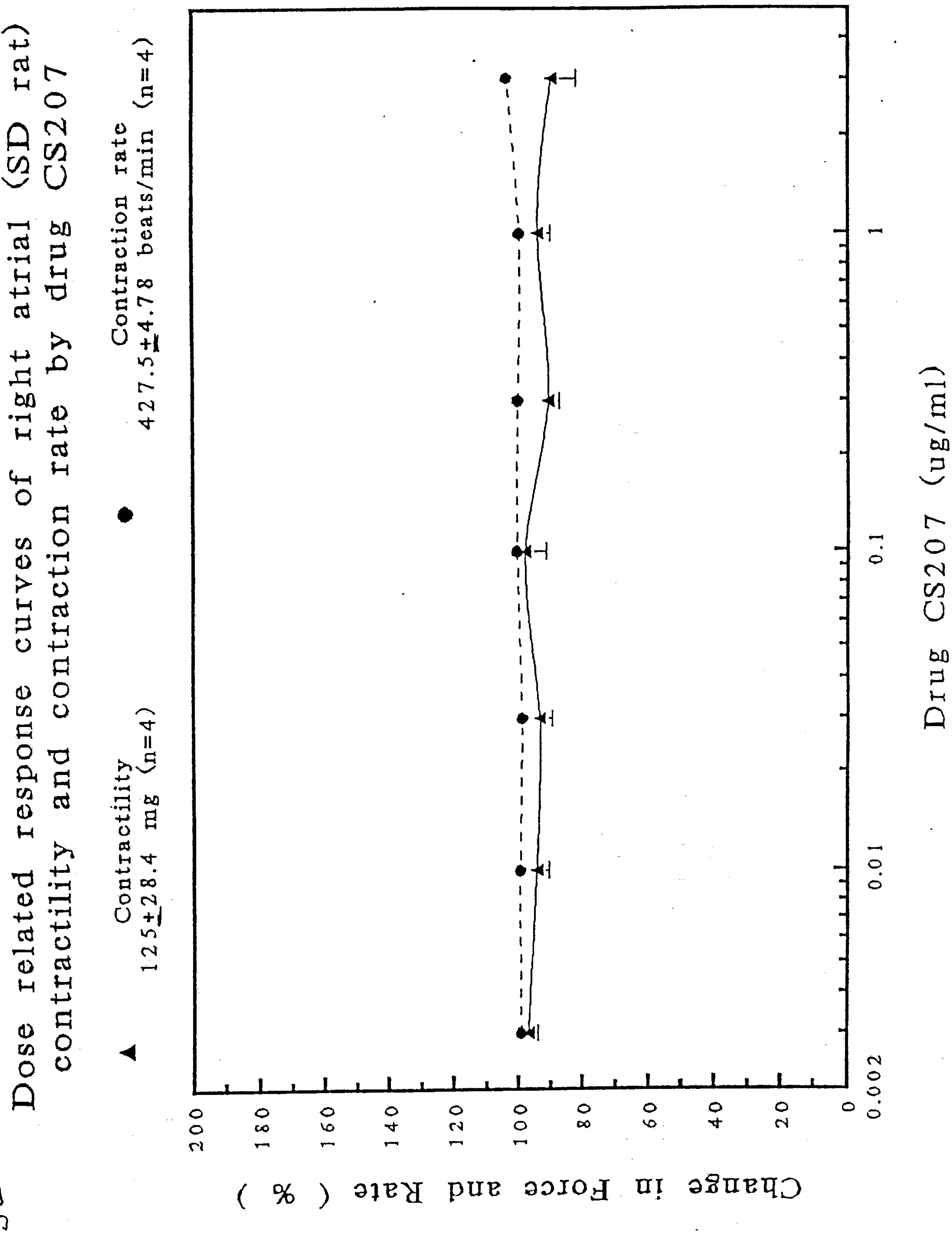


Fig 50

Fig. 51

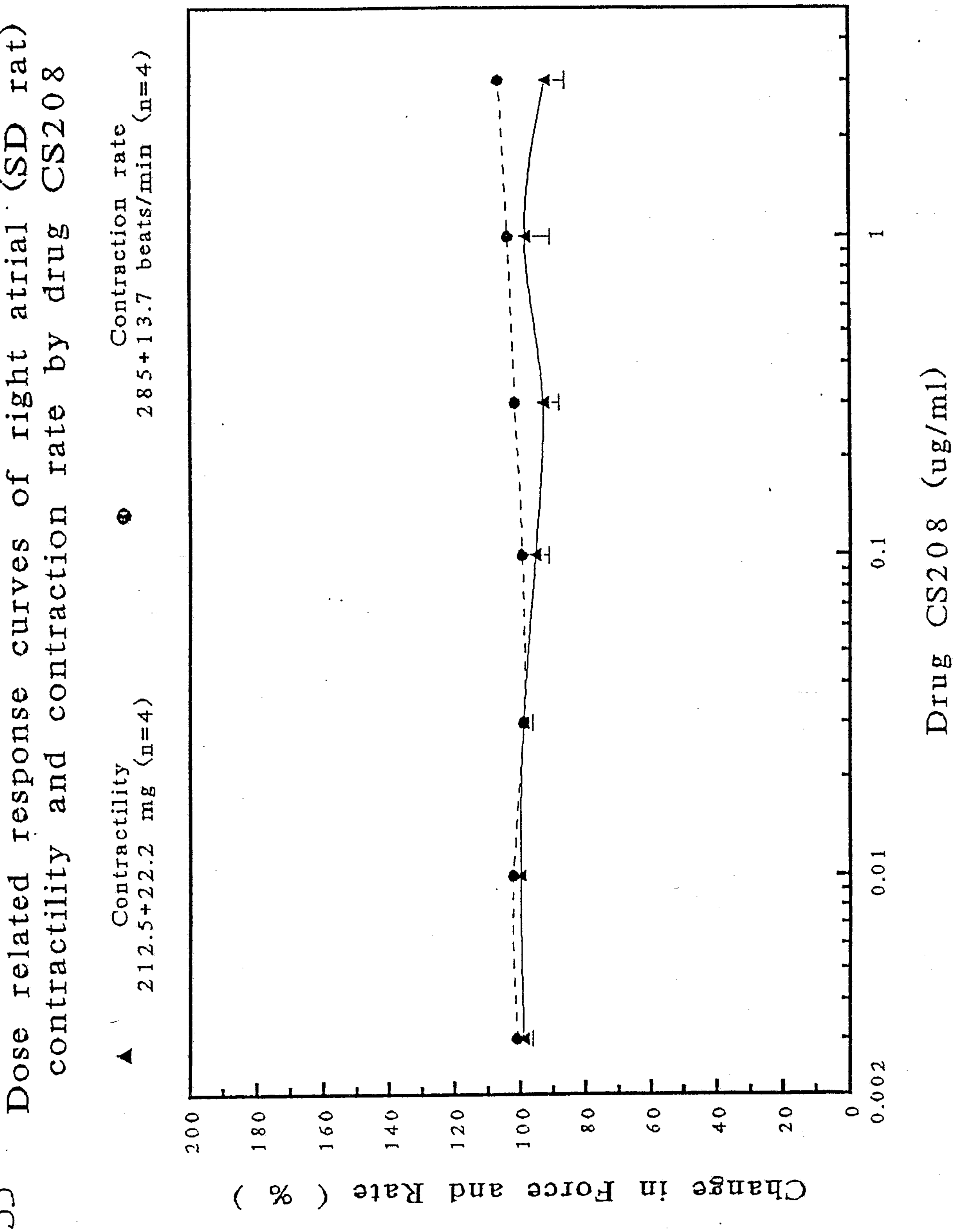
Dose related response curves of right atrial (SD rat) contractility and contraction rate by drug CS206





Dose related response curves of right atrial (SD rat) contractility and contraction rate by drug CS207

Fig. 52



Dose related response curves of right atrial (SD rat) contractility and contraction rate by drug CS208
▲ Contractility
212.5+22.2 mg (n=4)
● Contraction rate
285+13.7 beats/min (n=4)
Change in Force and Rate (%)
200
180
160
140
120
100
80
60
40
20
0
0.002
0.01
0.1
1
Drug CS208 (ug/ml)

Fig 53

Dose related response curves of right atrial (SD rat) contractility and contraction rate by drug CS209

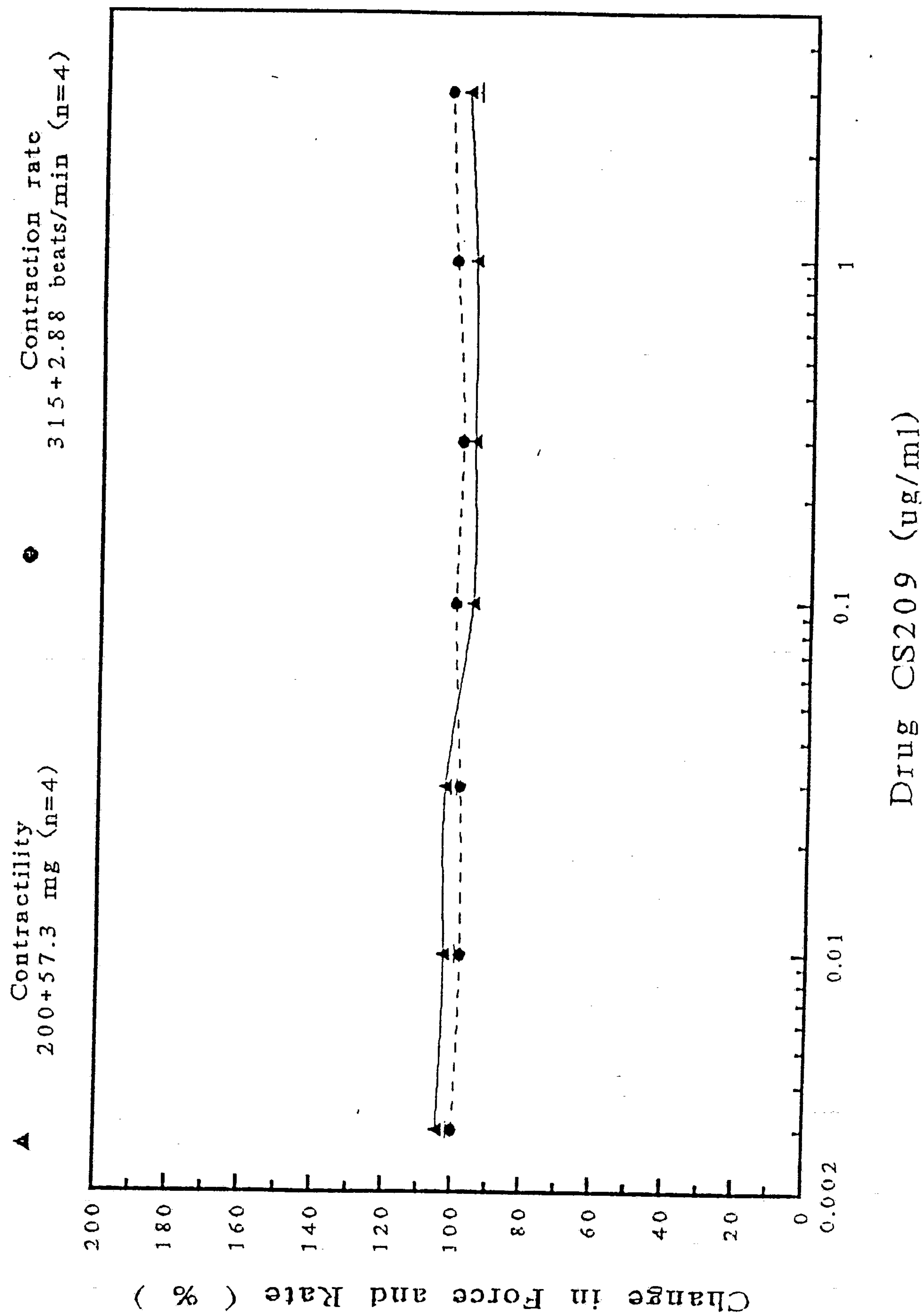


Fig 54

Dose related response curves of right atrial (SD rat) contractility and contraction rate by drug CS211

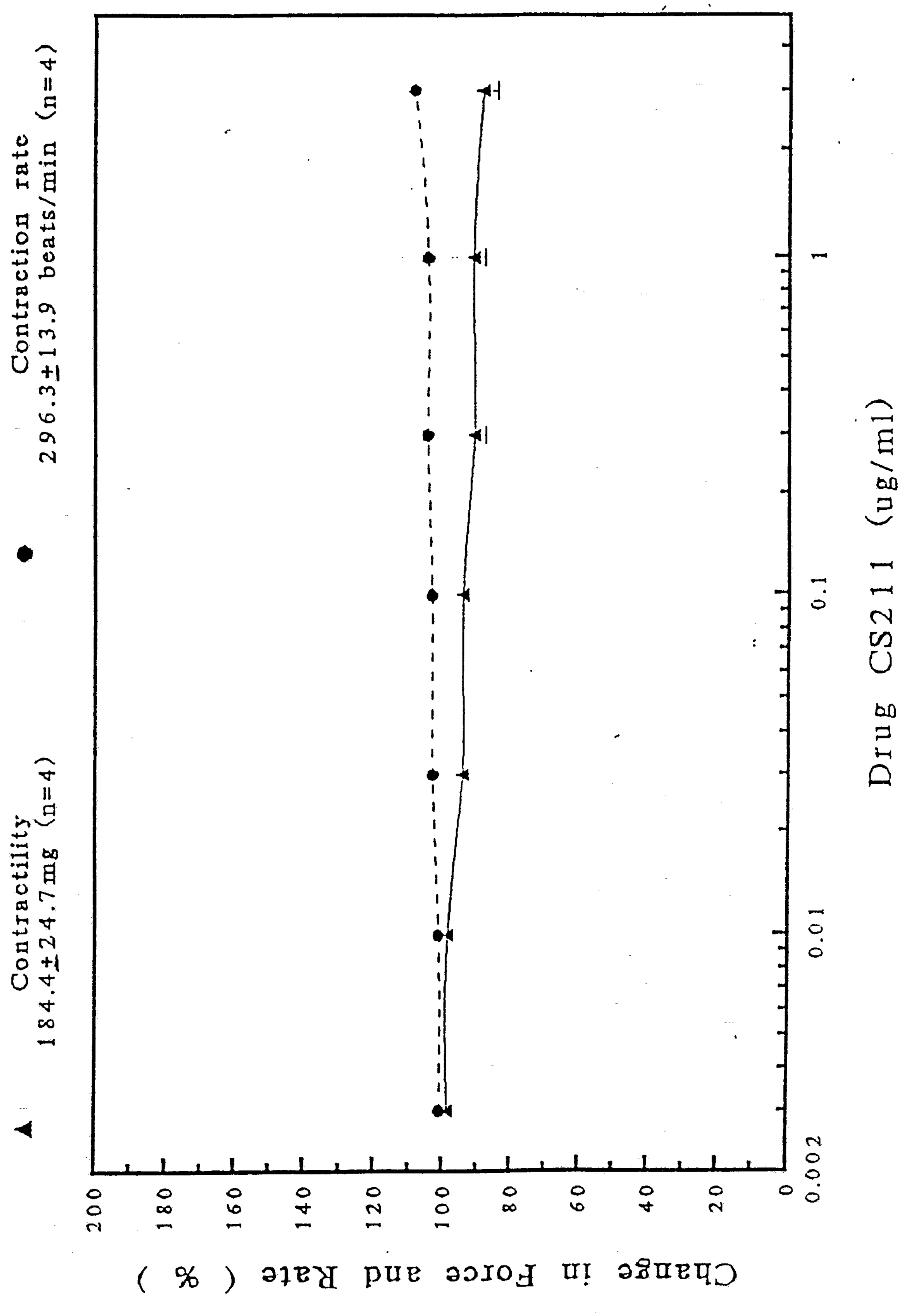


Fig 55

Dose related response curves of right atrial (SD rat) contractility and contraction rate by drug CS212

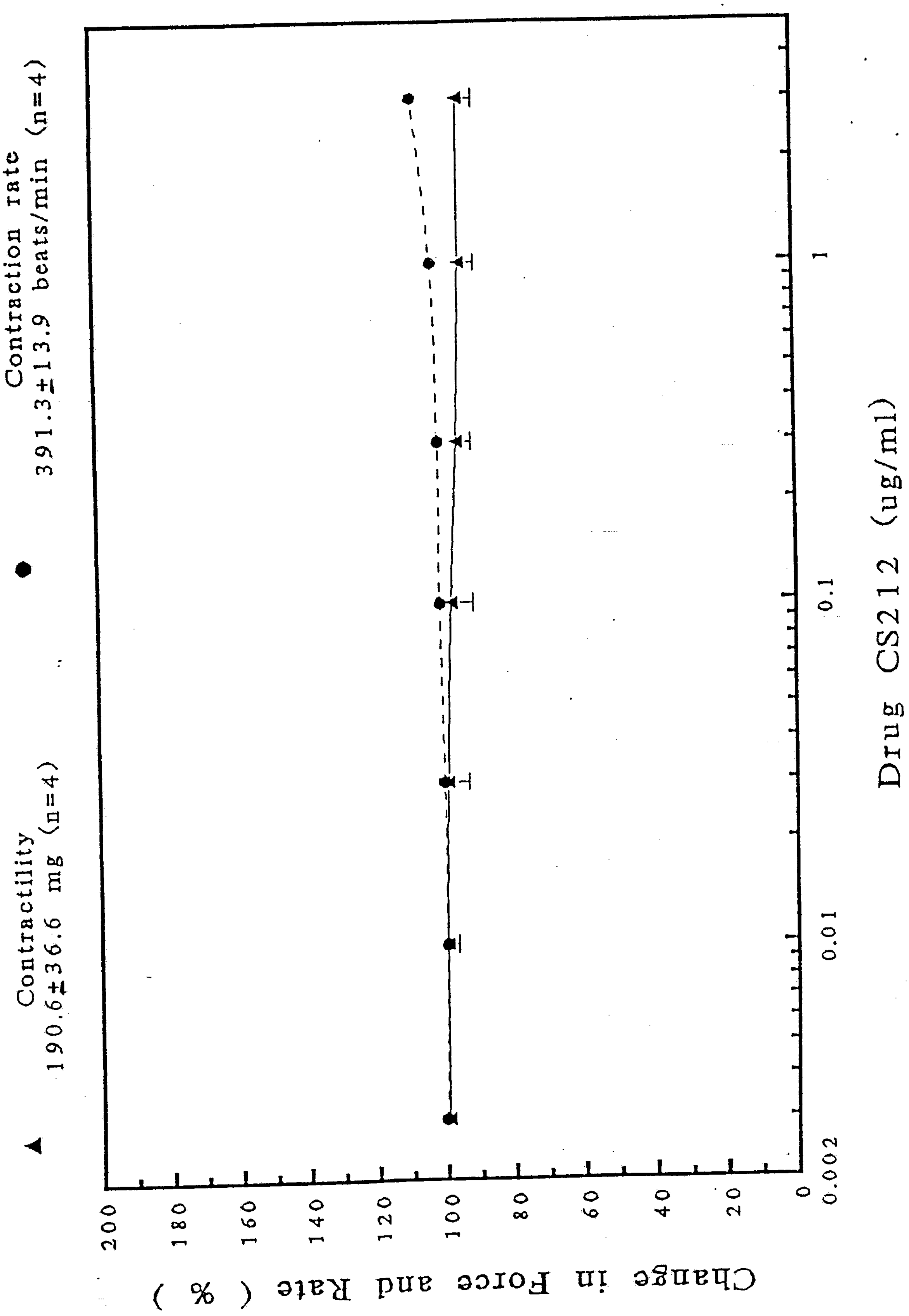


Fig. 56

Dose related response curves of right atrial (SD rat) contractility and contraction rate by drug CS213

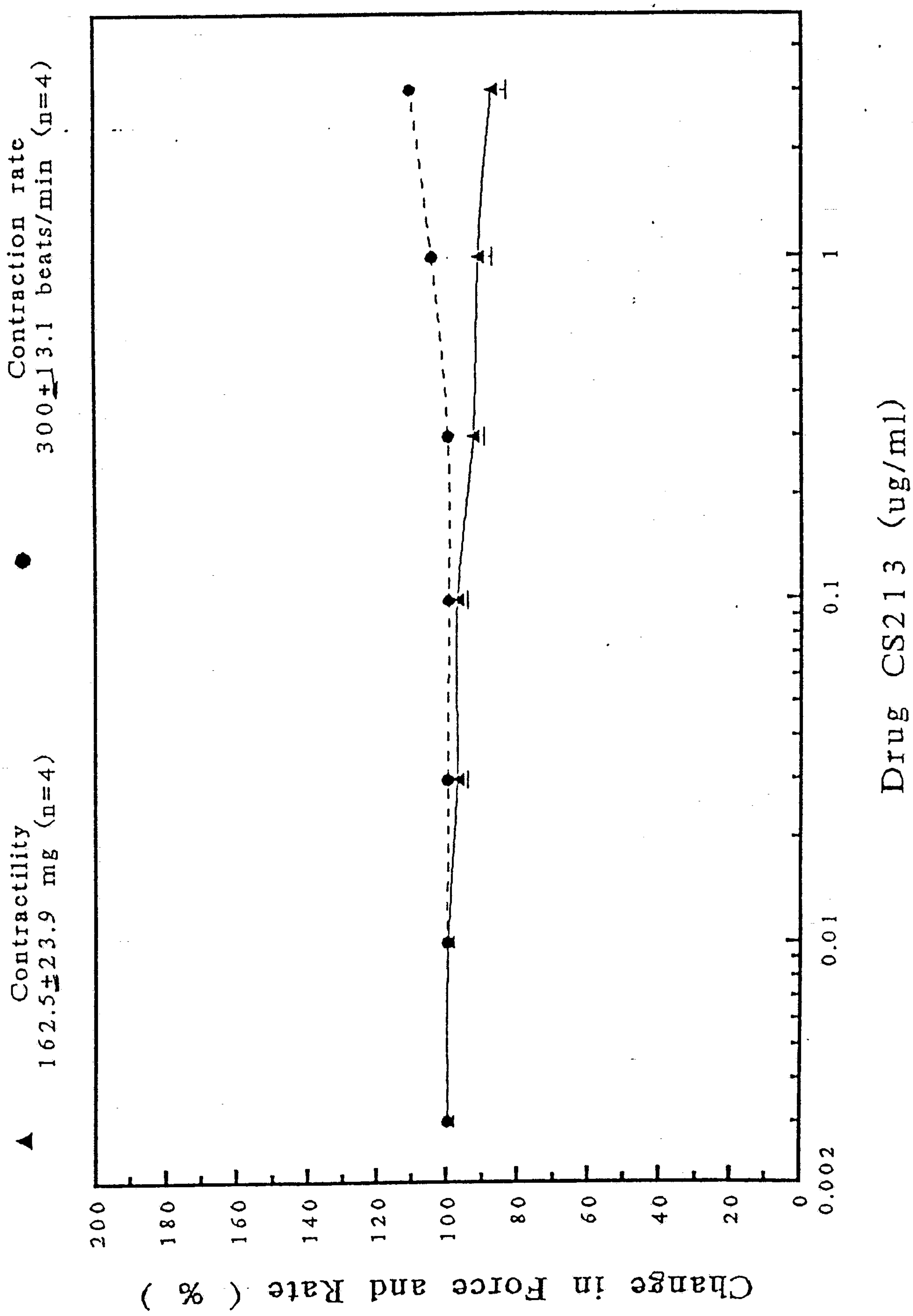


Fig 57

Dose related response curves of right atrial (SD rat) contractility and contraction rate by drug CS214

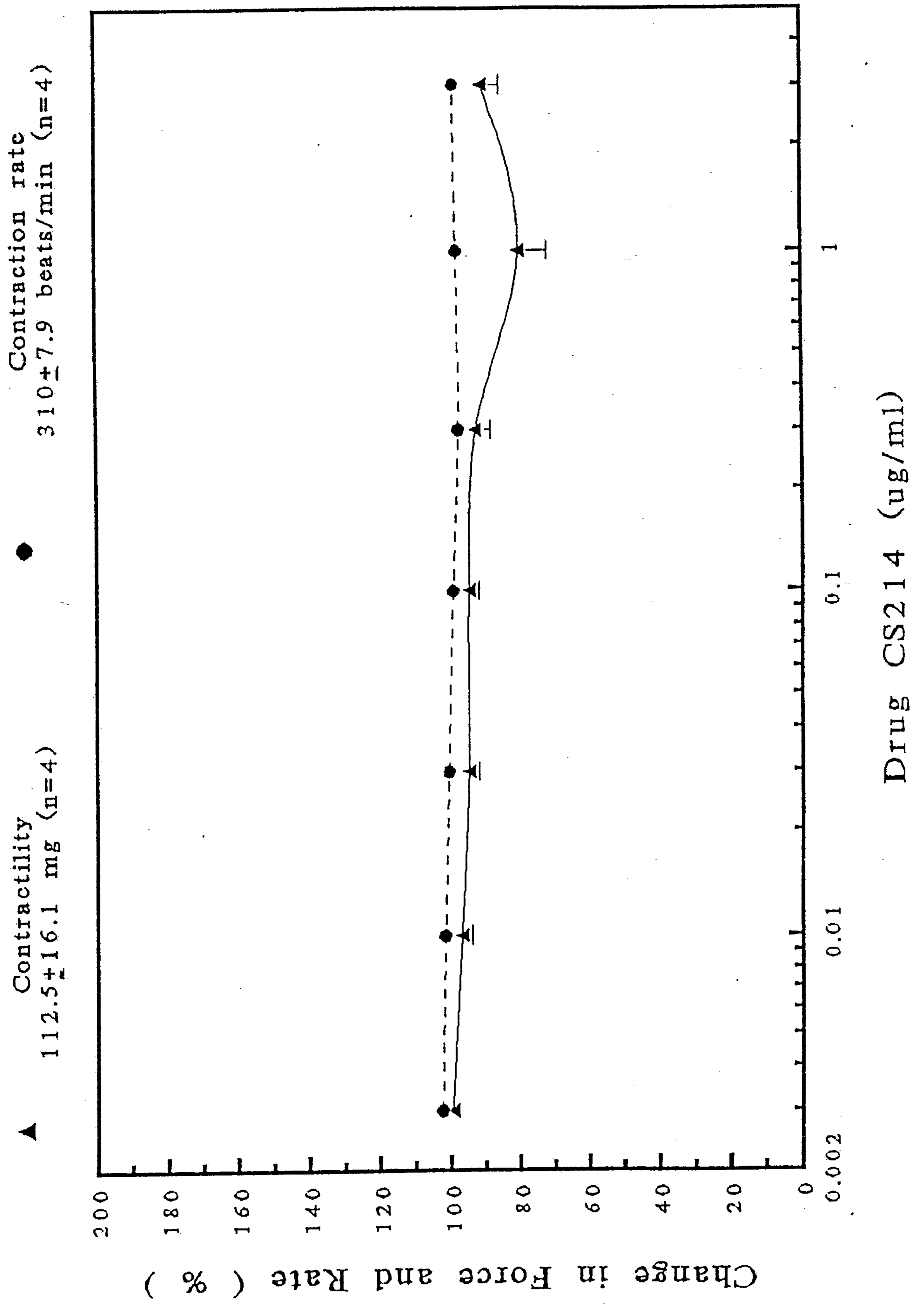


Fig. 58

Fig 59

Dose related response curves of right atrial (SD rat) contractility and contraction rate by drug CS215

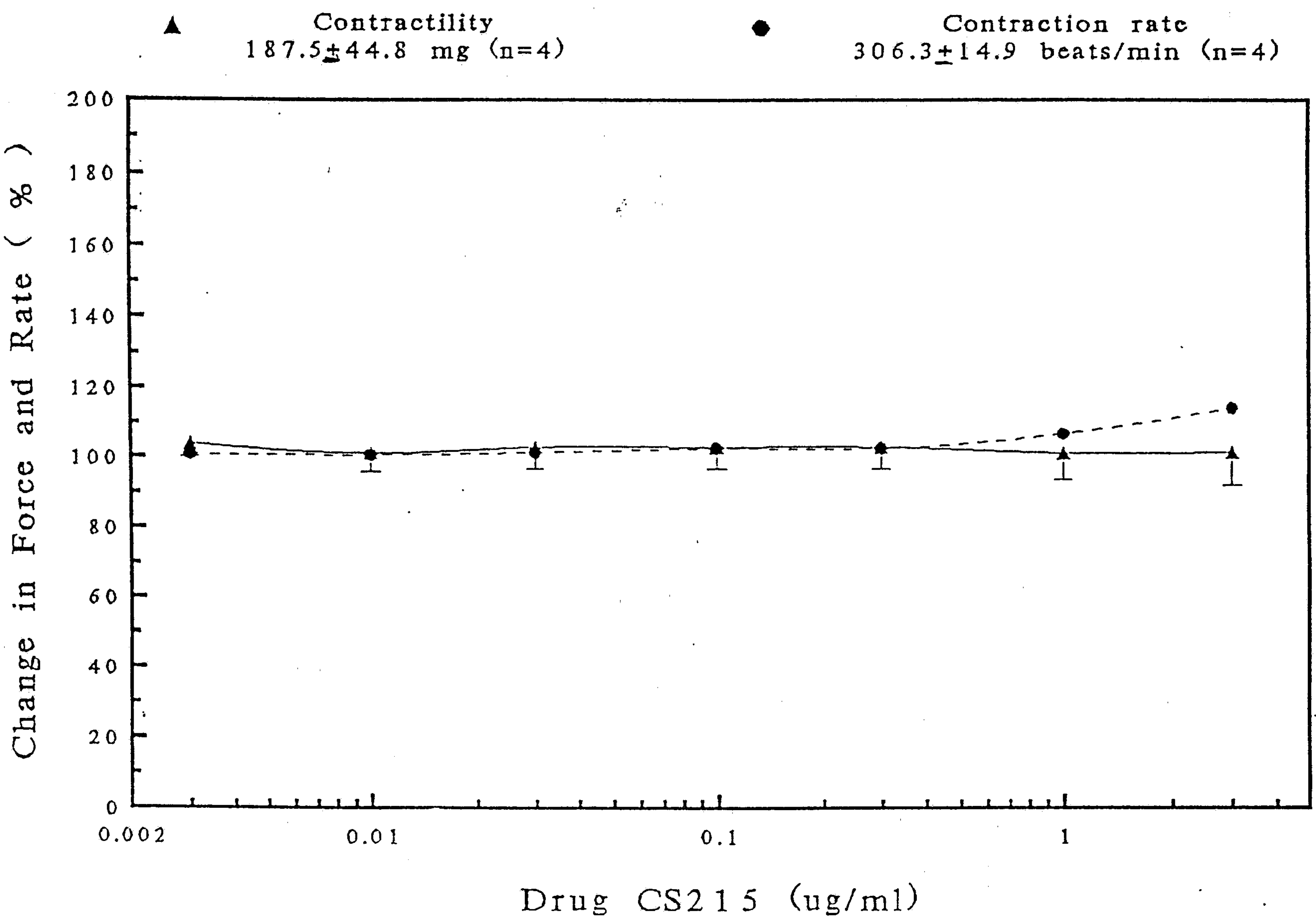
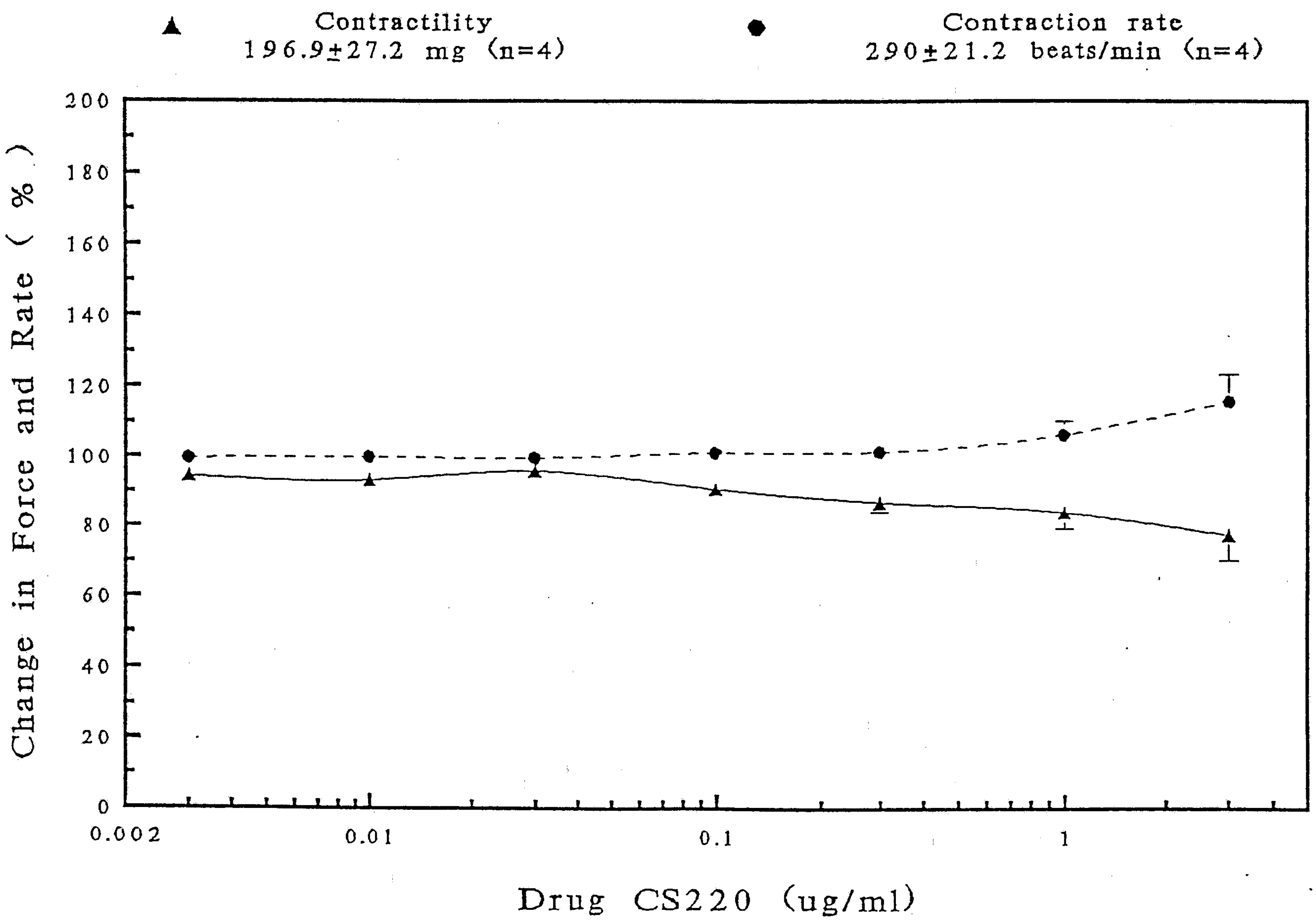


Fig. 60

Dose related response curves of right atrial (SD rat) contractility and contraction rate by drug CS220



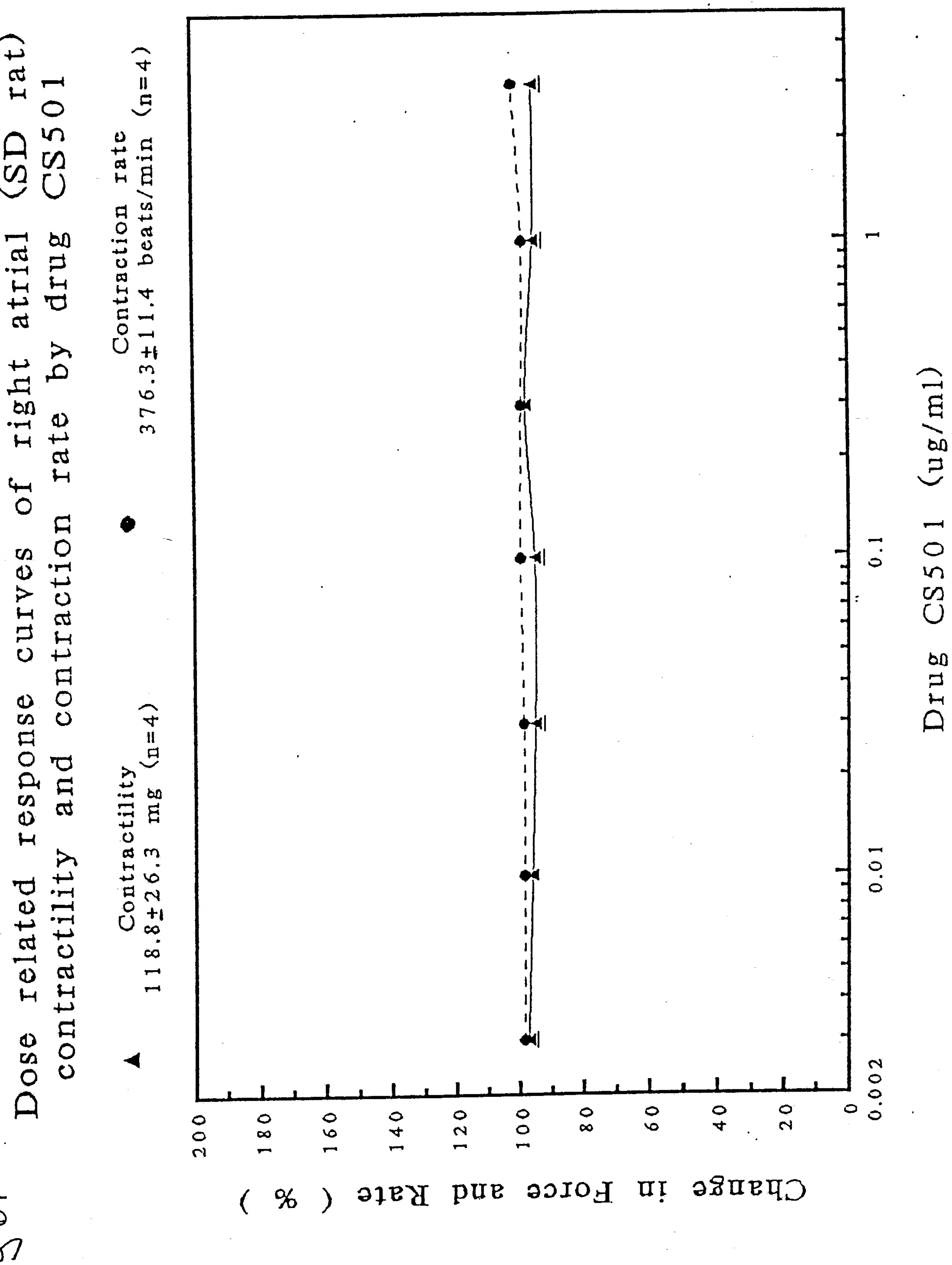

Dose related response curves of right atrial (SD rat)
contractility and contraction rate by drug CS501
Contractility
118.8±26.3 mg (n=4)
Contraction rate
376.3±11.4 beats/min (n=4)
Change in Force and Rate (%)
200
180
160
140
120
100
80
60
40
20
0
0.002
0.01
0.1
1
Drug CS501 (ug/ml)

Fig 61

Dose related response curves of right atrial (SD rat) contractility and contraction rate by drug CS503

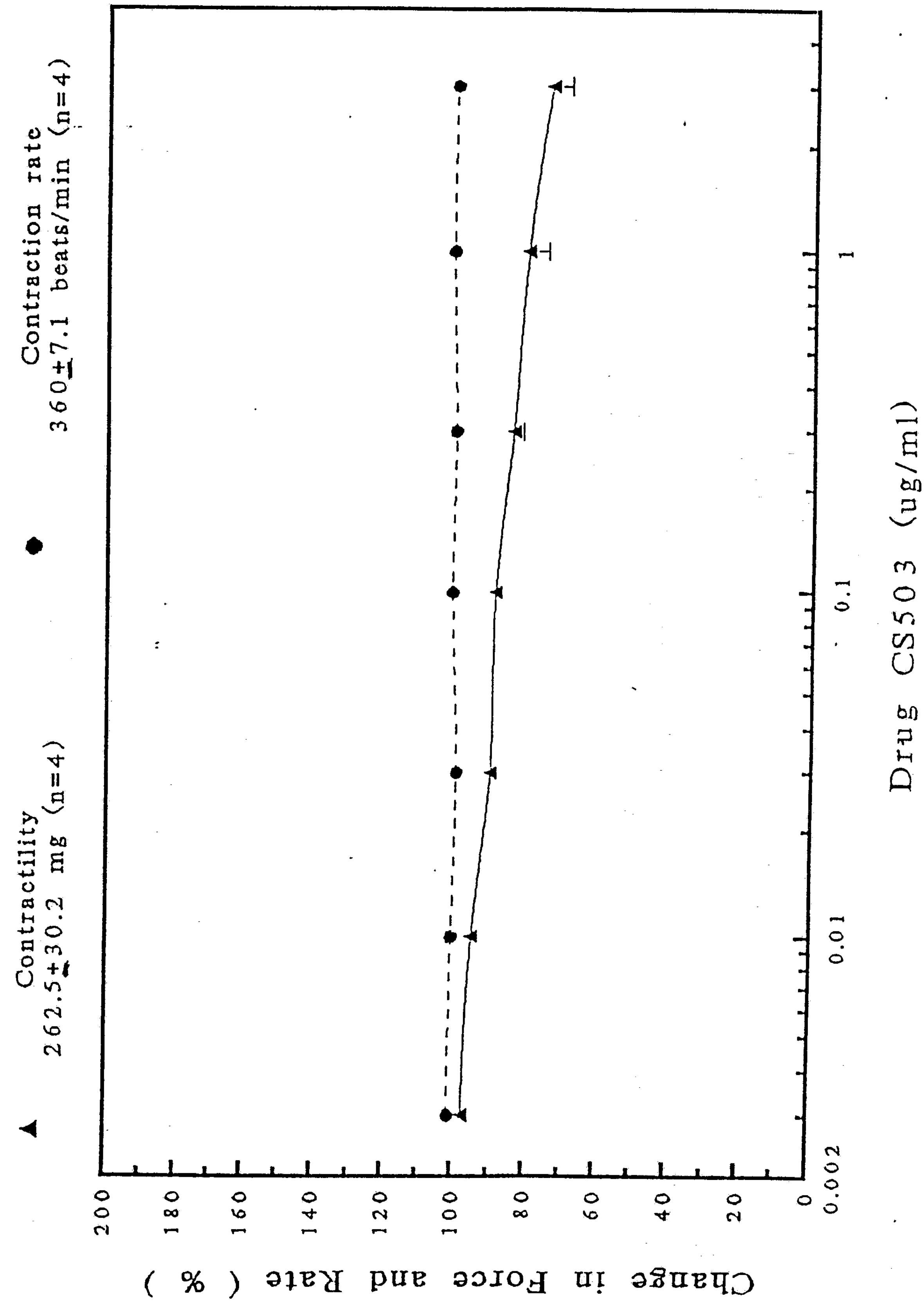


Fig 62

Dose related response curves of right atrial(SD rat) contractility and contraction rate by drug CS1001 CS2001

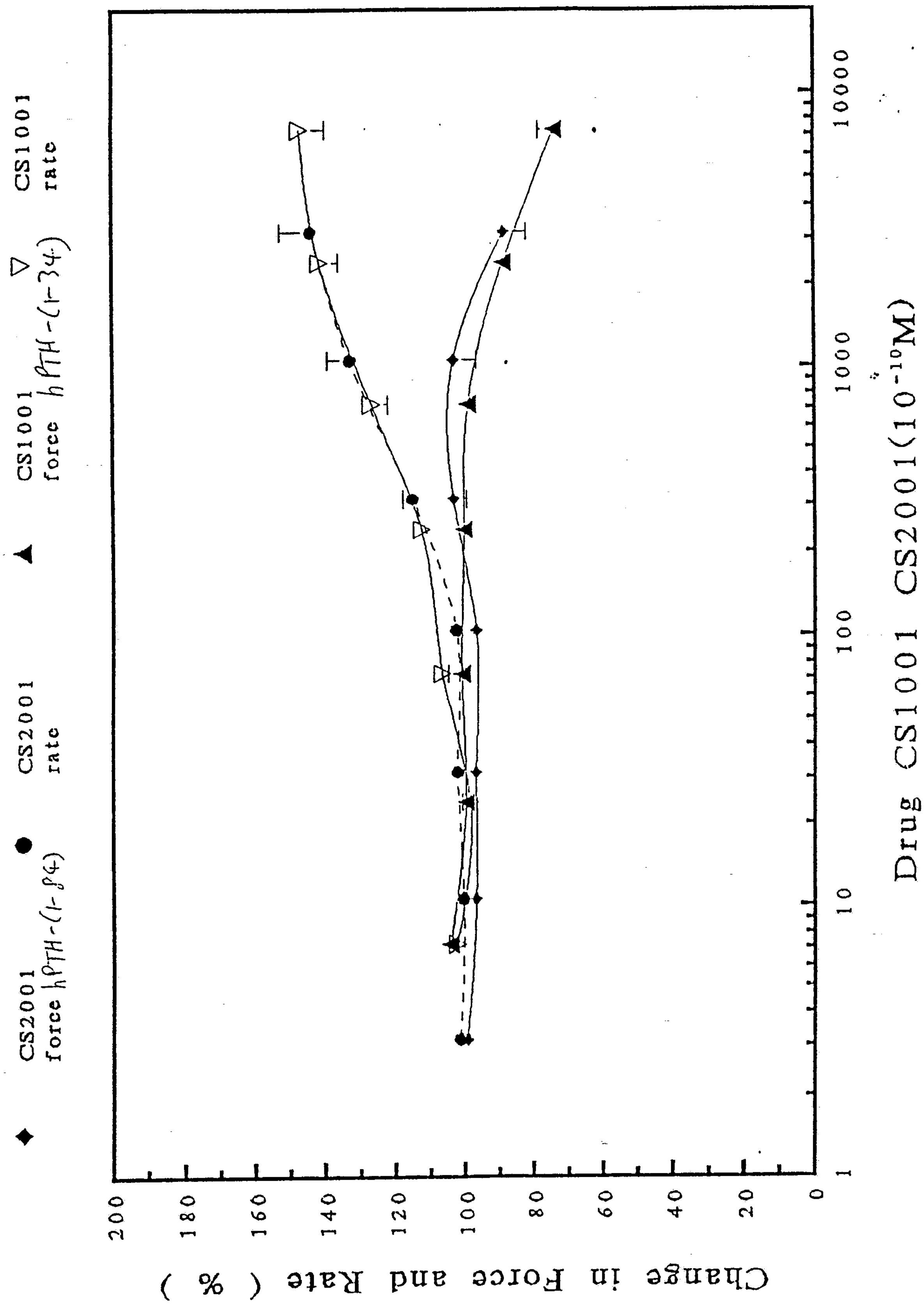


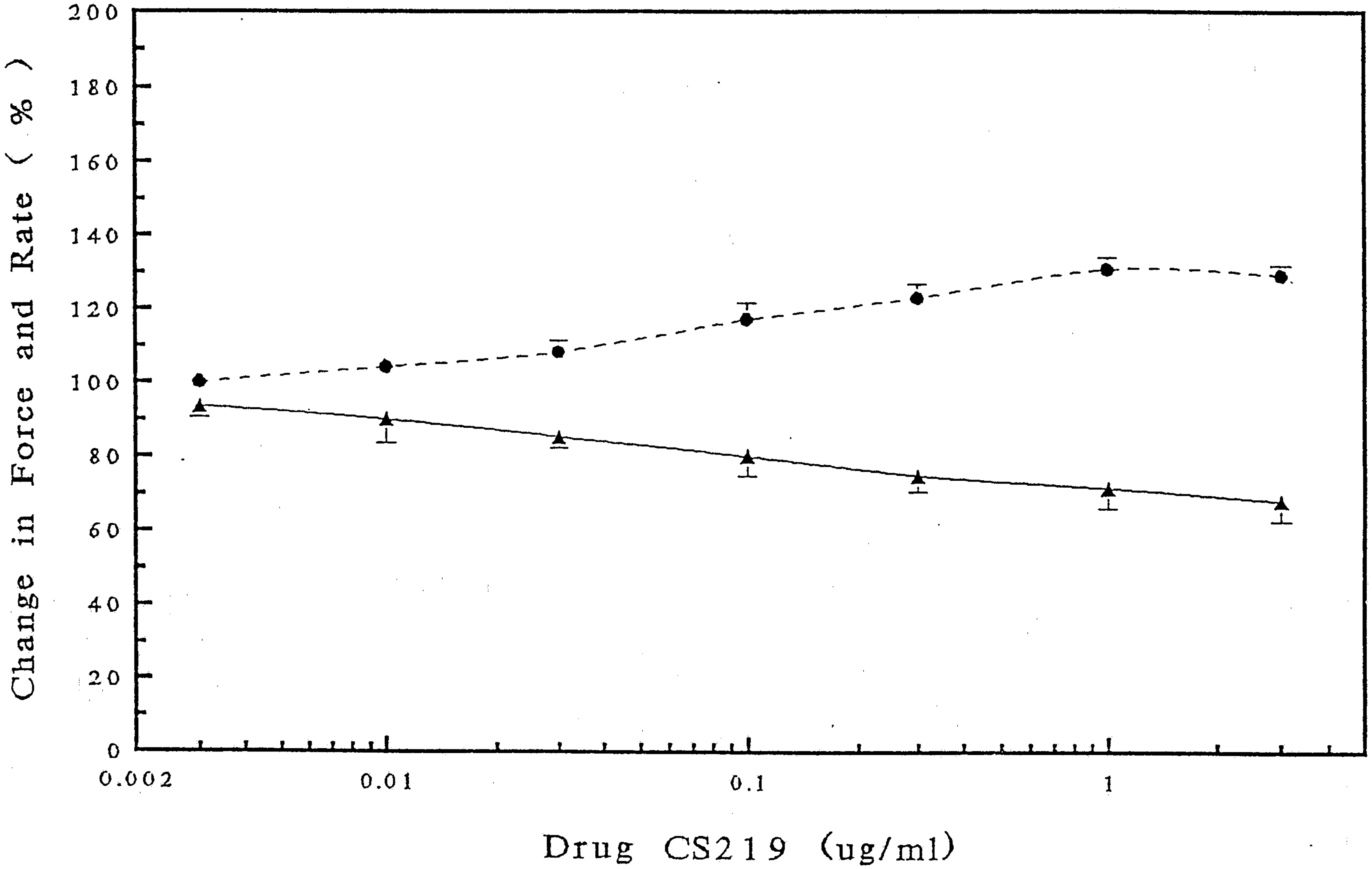
Fig 63

Fig 64

Dose related response curves of right atrial (SD rat) contractility and contraction rate by drug CS219 [Ile^{23}]-hPTH-(1-34)

▲ Contractility 256.3±49.3 mg (n=4)

● Contraction rate 312.5±11.3 beats/min (n=4)



Dose related response curves of right atrial (SD rat) contractility and contraction rate by drug CS218

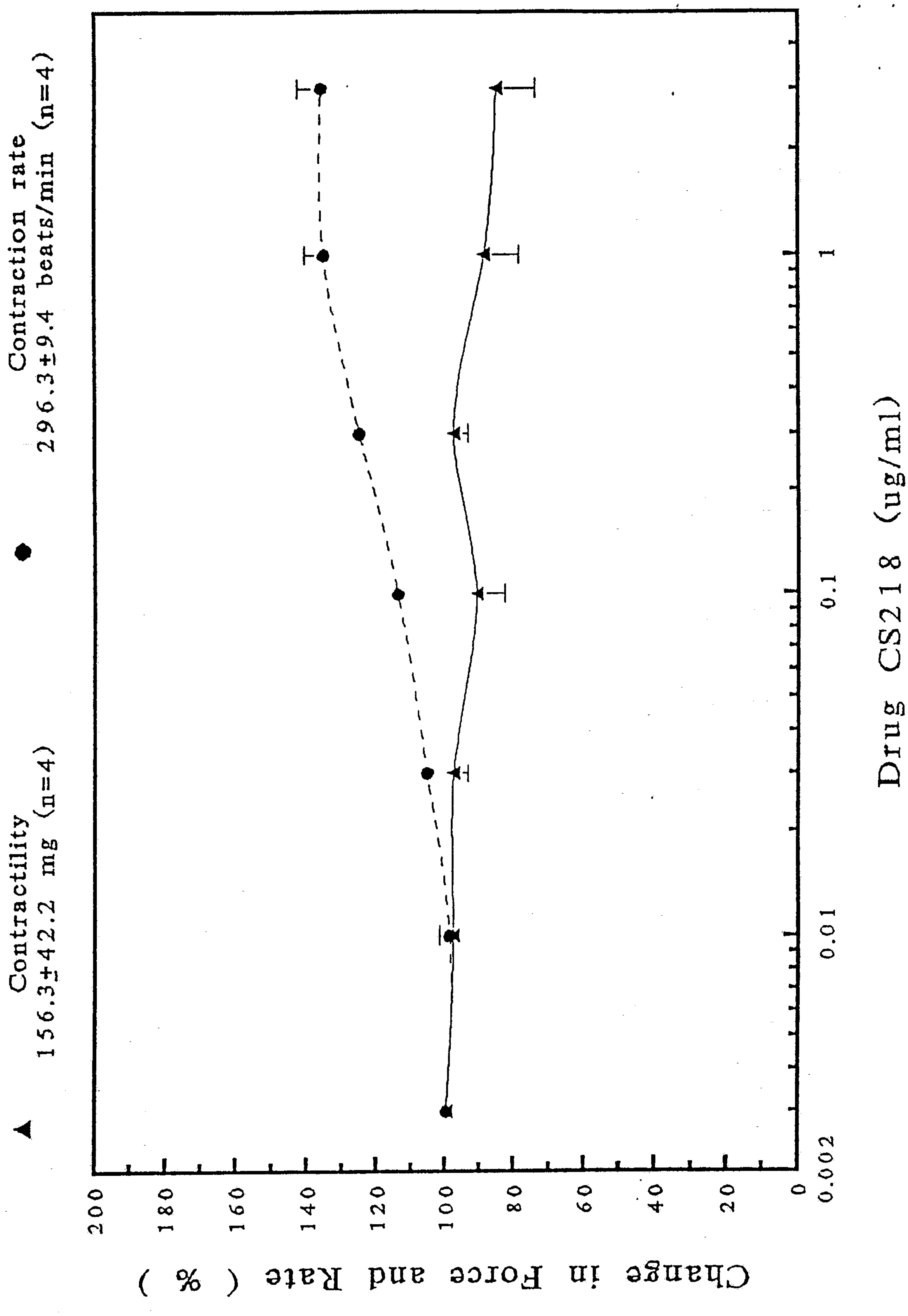


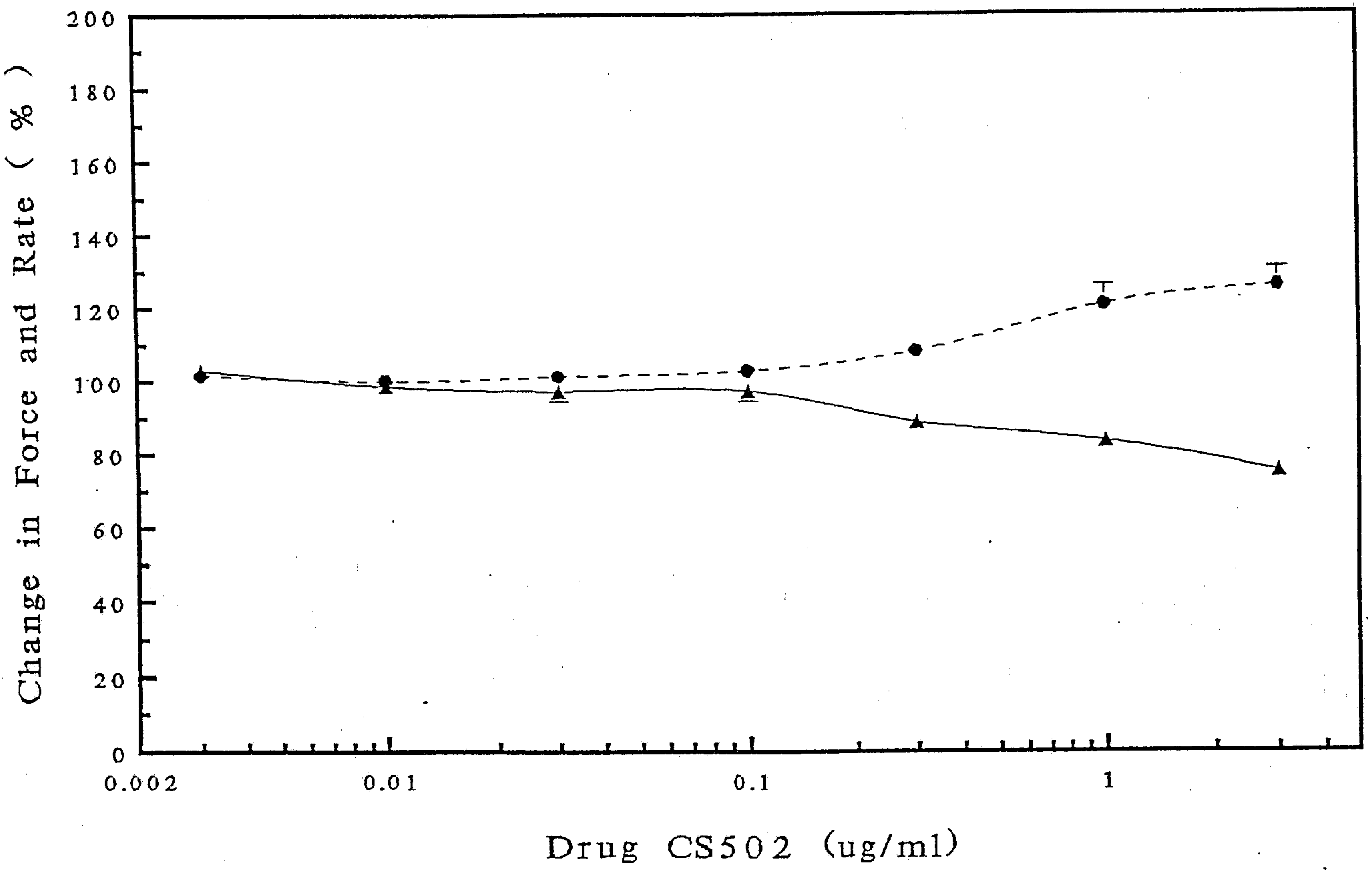
Fig 65

Fig 66

Dose related response curves of right atrial (SD rat) contractility and contraction rate by drug CS502 (Leu^{23}) hPTH-(1-34)

▲ Contractility 190.6±23.6 mg (n=4)

● Contraction rate 283.8±12.5 beats/min (n=4)



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/08478

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/02, 37/36; C07K 7/10
US CL :530/324, 399; 514/12
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2,21

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS (Parathyroid and Osteoporosis)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US,A, 4,771,124 (ROSENBLATT et al.) 13 September 1988. See the entire document particularly the abstract.	1-36
Y	US,A, 4,833,125 (NEER et al) 23 May 1989, see the entire document particularly the abstract.	1-36
Y	US,A, 4,086,196 (TREGEAR) 25 April 1978, see the entire document particularly the abstract.	1-36

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 DECEMBER 1992	12 JAN 1993

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