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(54) Title: THERAPEUTIC COMPOUNDS, COMPOSITIONS AND USES THEREOF

(57) Abstract

The use of calcitonin gene-related peptide in achieving the controlled reduction of blood pressure while maintaining cerebrovascular blood supply is described. This activity of CGRP makes it especially suitable for use in the treatment of malignant hypertension and hypertension following cardiac surgery.

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Therapeutic Compounds, Compositions and Uses Thereof

Field of the Invention

This invention relates to a new medical use for certain compounds and pharmaceutical compositions containing them. In particular it relates to the use of calcitonin gene-related peptide (CGRP) in achieving the controlled reduction of blood pressure while maintaining cerebrovascular blood supply.

Background to the Invention

Calcitonin gene-related peptide is a product of the calcitonin gene expression system. Alternative processing of RNA transcribed from the calcitonin gene leads to the production in neuronal tissue of CGRP, a 37 amino acid peptide. CGRP has been discovered in a number of species including rats, chickens and humans. The CGRPs are a very closely related group of compounds differing from each other by no more than a few amino acids. To date CGRP has been described as being principally of use in the treatment of hypertension owing to its properties on the cardiovascular system where it has been found to cause vasodilatation and to lower blood pressure. CGRP has also been postulated to play a role in calcium regulation and gastric acid secretion. We have now discovered that CGRP is useful in achieving the controlled reduction of blood pressure while maintaining cerebrovascular blood supply.

The blood pressure may become raised in association with a number of disease states. Malignant hypertension is accompanied by a sudden rapid rise in blood pressure to extremely high levels e.g. of up to 340/280mmHg. If untreated, the majority of subjects will die within a relatively short period of time. One of the commonest causes of malignant hypertension is as a secondary effect of a peptide secreting tumour.

Current treatments involve the use of for example diuretics or β-blockers and α-blockers such as phentolamine and vasodilators such as nitroprusside . These treatments have associated side effects including anxiety, dizzines and lethargy. Use of these agents results in a sudden dramatic fall in blood pressure which causes cerebral insufficiency which can in extreme cases lead to brain ischaemia.

Hypertension is commonly observed following cardiac surgery. Manipulation of the heart triggers reflex mechanisms which lead to an overflow to the sympathetic nervous system. This in turn leads to vasoconstriction which results in an increase in blood pressure.

In order to be useful in the treatment of malignant hypertension and hypertension following cardiac surgery it is essential that the therapeutic agent is able to selectively vasodilate the blood vessels of the target vascular bed, such that the blood supply is increased only at the desired site and a controlled drop in blood pressure is achieved. A further essential requirement is that the blood supply should be increased while the cerebrovascular blood supply is maintained.

We have found that it is possible to achieve the required selectivity of site of action and the desired maintenance of cerebrovascular blood supply and effect a controlled reduction in blood pressure by administration of an appropriate amount of CGRP.

Summary of the Invention

Accordingly in a first aspect the invention provides CGRP for use in the controlled reduction of blood pressure accompanied by the maintenance of cerebrovascular blood supply.

As used herein the term CGRP, in addition to naturally occurring CGRPs, includes also biologically active fragments, analogues and derivatives thereof which have the characteristic blood supply affecting properties of CGRP: i.e. which preferentially act on the target vascular bed resulting in a controlled reduction in blood pressure without substantially affecting cerebrovascular blood supply. The CGRPs, fragments, analogues and derivatives may be naturally occurring or may be produced chemically e.g. by chemical modification, cleavage, or synthesis or they may be produced by employing recombinant DNA techniques. The fragments, analogues and derivatives may include non-peptide compounds as well as peptide compounds. For examples of suitable CGRP analogues see International Patent Application No. PCT/GB89/01249 filed 20th October 1989. The CGRP may comprise an animal CGRP, e.g. rat or chicken CGRP, though is preferably human calcitonin gene-related peptide (hCGRP). Human calcitonin gene-related peptide exists in at least two forms known as alpha hCGRP, for instance as described in the US Patent No. 4549986, and beta hCGRP, for instance as described in published European Patent Application No. EP 188400A. As used herein, the term 'hCGRP' is used to denote α- and β-hCGRP. The use of α-hCGRP is especially preferred.

CGRP is particularly useful in the treatment of hypertension in human subjects associated with malignant hypertension and following cardiac surgery.

According to a second aspect of the invention we provide a method of treatment of a human subject suffering from malignant hypertension or hypertension following cardiac surgery which comprises administering an effective amount of CGRP to the subject.

Typically the amount of CGRP used is an amount of CGRP which is effective in reducing blood pressure in a controlled way whilst maintaining the cerebrovascular blood supply.

In a third aspect the invention provides a pharmaceutical composition comprising CGRP for use in the treatment of malignant hypertension or hypertension following cardiac surgery.

In a fourth aspect the invention provides a pharmaceutical composition in unit dosage form, each unit dose comprising an amount of CGRP which acts to reduce blood pressure in a controlled way whilst maintaining the cerebrovascular blood supply in combination with a pharmaceutically acceptable carrier, excipient or diluent.

The pharmaceutical composition according to the fourth aspect of the invention preferably contains 0.01 to 45µg CGRP, preferably 0.08µg to 35µg CGRP, more preferably from 5 to 35µg CGRP and most preferably from 5 to 25µg CGRP.

In a fifth aspect the invention provides a process for the production of a pharmaceutical composition according to the fourth aspect of the invention comprising bringing into association with a pharmaceutically acceptable carrier, excipient or diluent aliquot amounts of CGRP sufficient to reduce blood pressure in a controlled way whilst maintaining the cerebrovascular blood supply to provide unit doses.

In a sixth aspect the invention provides the use of CGRP for the manufacture of a medicament for the controlled reduction in blood pressure whilst maintaining cerebrovascular blood supply.

In a preferred embodiment of the sixth aspect of the invention the invention provides CGRP for the manufacture of a medicament for the treatment of malignant hypertension or hypertension following cardiac surgery.

In a seventh aspect the invention provides a drug for therapy of malignant hypertension or hypertension following cardiac surgery comprising a CGRP as an active ingredient.

In an eighth aspect the invention provides a method for the treatment of malignant hypertension or hypertension following cardiac surgery which comprises administering to a patient a CGRP.

In a ninth aspect the invention provides a controlled reducer of blood pressure and maintainer of cerebrovascular blood supply comprising CGRP.

Pharmaceutical compositions for use according to the present invention may be formulated in conventional manner, optionally with one or more physiologically acceptable carriers diluents or excipients.

Compounds for use according to the present invention may be formulated for oral, buccal, parenteral or rectal administration or in a form suitable for nasal administration or administration by inhalation or insufflation.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g. pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g. lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g. magnesium stearate, talc, or silica); disintegrants (e.g. potato starch or sodium glycollate); or wetting agents (e.g. sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such

liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents; emulsifying agents; non-aqueous vehicles; and preservatives. The preparations may also contain buffer salts, flavouring, colouring and sweetening agents as appropriate.

Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

The CGRP may be formulated for parenteral administration by injection e.g. by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilising and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile pyrogen-free water, before use.

The CGRP may also be formulated in rectal compositions such as suppositories or retention enemas, e.g. containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the CGRP may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation or by intramuscular injection.

For nasal administration or administration by inhalation the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation

from pressurised packs or a nebuliser, with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas.

The compositions may contain one or more unit dosage forms containing the active ingredient. The pack or dispenser device may be accompanied by instructions for administration.

The dose at which CGRP will be administered to man will be such that the blood pressure is reduced in a controlled way whilst maintaining the cerebrovascular blood supply. The precise dose of CGRP will depend upon the route of administration, the potency of the CGRP and the body weight and pathology of the patient. The important factor is believed to be the concentration of CGRP which is present at the target vascular bed. On an individual patient basis the dose of the CGRP which should be administered to cause the desired effect may be determined by administering a low dose of CGRP for 10 - 20 minutes and then increasing the dose every 10 - 20 minutes until the desired effect is seen. A CGRP may be administered to an average 70kg man by IV infusion at doses in the range 0.01-32ng/kg/min, preferably in the range 0.06 to 24ng/kg/min, more preferably in the range 2 to 24ng/kg/min, and most preferably in the range 2-16ng/kg/min. For example, α-CGRP and β-CGRP may be administered to an average 70kg man by IV infusion at doses in the range 2ng/kg/min to 16ng/kg/min over a time period of 20 minutes. In some cases it may be desirable to infuse the patient with CGRP for longer time periods e.g. for up to or greater than 1 hour, e.g. for 12 hours or for more than 24 hours.

CGRP for use in the present invention may be obtained using recombinant DNA technology as described in British patent No. 2141430B and published European Patent Application No. EP-A-188400. Alternatively the CGRP may be produced by chemical synthesis using conventional techniques well known in the art, see for example published European Patent Application No. EP-A-188400.

CGRP is particularly useful in the treatment of malignant hypertension and hypertension following cardiac surgery since it shows the desired profile of activity i.e. it reduces the blood pressure in a controlled way whilst maintaining the cerebrovascular blood supply.

The CGRP may be tested for its ability to reduce blood pressure without affecting cerebrovascular blood supply in animals and humans using the Doppler technique as described in the examples hereinafter. The effect of the CGRP on blood pressure and cerebrovascular blood supply may be measured using conventional techniques.

Brief Description of Drawings

The invention is further illustrated in the following non-limiting examples and with reference to the following figures in which:

Figure 1: Shows the effect on heart rate, mean arterial pressure and carotid Doppler shift during and after infusion of CGRP at 0.6nmol h^{-1}

αhCGRP

βhCGRP

Figure 2: Shows the effect on heart rate, mean arterial pressure and vascular resistance during and after infusion of CGRP at 0.6nmol h^{-1}

αhCGRP

βhCGRP

Figure 3: Shows the effect on heart rate, mean arterial pressure, Doppler shift and vascular resistance of CGRP analogue CB007 infused for 60 min at 0.6nmol/h

Figure 4: Shows the effect on heart rate, mean arterial pressure, Doppler shift and vascular resistance of CGRP analogue CB008 infused for 60 min at 0.6nmol/h

Figure 5: Shows the effect on heart rate, mean arterial pressure, Doppler shift and vascular resistance of CGRP analogue CB009 infused for 60 min at 0.6nmol/h

Figure 6: Shows the effect on heart rate, mean arterial pressure, Doppler shift and vascular resistance of CGRP analogue CB010 infused for 60 min at 0.6nmol/h

Figure 7: Shows the effect on heart rate, mean arterial pressure, Doppler shift and vascular resistance of CGRP analogue CB011 infused for 60 min at 0.6nmol/h

Figure 8: Shows the effect on heart rate, mean arterial pressure, Doppler shift and vascular resistance of CGRP analogue H7030 infused for 60 min at 0.6nmol/h

Figure 9: Panel A: Shows the effect on internal carotid volume of infusion of human αCGRP at 8ng/kg/min

Panel B: Shows the effect on mean systolic blood pressure of infusion of human αCGRP at 8ng/kg/min

Description of Specific Embodiments

Example 1.

This series of experiments was designed to look at the effect in rats of infusion of human CGRP on mean arterial pressure, heart rate and blood flow through the right and left carotid arteries.

Male, Wistar rats (from Bantin and Kingman) were housed in groups of 4 in cages with solid floors and sawdust covering, and with free access to food (Labsure 41B) and water. They were kept in the animal unit for at least 2 weeks before being used, to ensure they were in good health before entering the experimental protocol. At the time of operation animals weighed between 330-420g. Under anaesthesia (sodium methohexitone 60 mg kg^{-1} i.p., supplemented) pulsed Doppler probes (Haywood et al., Am. J. Physiol 241 H273-H278 1981) were implanted around left renal and superior mesenteric arteries and the distal abdominal aorta (below the level of the ileocaecal artery), or, in other animals, around both common carotid arteries. Care was taken not to damage paravascular nerve bundles; the latter were separated from the vessels before the probes were implanted around them. (In the case of animals with bilateral common carotid probes, reflex heart rate (HR) changes to increases or decreases in mean blood pressure (MBP) were normal and there were no signs of ptosis.) Probe wires were tunnelled subcutaneously and exited through a small skin incision at the back of neck where they were anchored by a tight ligature with their ends lying free. At least 1 week after operation all animals that were growing normally, grooming etc., and with acceptable signals from all 3 probes (signal:noise ratio > 20:1) were briefly re-anaesthetized (sodium methohexitone 40mg kg^{-1}) and had intravascular catheters implanted and the probe wires soldered into a microconnector clamped in a harness fitted to the rat. The same harness has a flexible spring attached to it and the catheters ran through the spring for protection. The probe microconnector was plugged into a lead that was taped to the spring and the whole assembly was attached to a

counterbalanced, universally-jointed gantry. Thus the animal was not at all restricted in its movements, but, usually, was not able to get at the wires or catheters.

No measurements were made until the following day when at 0615h, continuous recordings of MBP, instantaneous HR, and renal, mesenteric and hindquarters, or bilateral common carotid, Doppler shift signals were begun. Phasic Doppler shift signals were monitored to ensure their acceptability and mean Doppler shift signals were obtained from them electronically. Changes in Doppler shift signal give a reliable index of changes in volume flow (Haywood et al., 1981; Wright et al., Hypertension 9 122-131 1987) and these were calculated as % of the baseline level. From MBP and mean Doppler shift, % changes in vascular resistance were calculated relative to the baseline level (Haywood et al., 1981).

The experimental protocol ran over 2 days and animals were randomized to receive human α or β-CGRP on the first experimental day. All animals received human α-CGRP on one day and human β-CGRP on the other day, both peptides being given as 60 min infusions. The infusate consisted of CGRP dissolved in isotonic saline containing 1% bovine serum albumin and 0.05% lactose. The amount of peptide needed to achieve the desired concentration was calculated from the stated peptide content of the lyophylizate.

Measurements were made at 0, 5, 10, 20, 30, 40, 50, 60, 65, 70, 80, 90, 100, 110 and 120 min., with the infusion running between 0 and 60 min. Data were analysed by two-way, non-parametric analysis of variance (Friedman's test). In the text where effects are referred to, the $P<0.05$, at least.

α- or β-CGRP infusion at 0.6nmol h^{-1}

a) Animals with renal, mesenteric and hindquarters probes.

Infusion of α-CGRP was accompanied by hypertension (5-65 min) and tachycardia (5-80 min). Renal (20-65 min) and mesenteric (5-70 min) flows were reduced and hindquarters flow was increased (5-70 min). There were renal (5-60 min) and hindquarters (5-70 min) vasodilatations, and a mesenteric vasoconstriction (5-70 min). After infusion, there was a significant bradycardia (100,120 min) and a secondary fall in MBP (120 min) accompanied by a renal vasodilatation (120 min), but no other changes.

Infusion of β-CGRP caused a tachycardia (5-65 min) similar to that seen with a α-CGRP, but the fall in MBP was less persistent (5-65 min). Renal flow was not significantly affected by β-CGRP, and the reduction in mesenteric flow (5-65 min) was not as prolonged as with α-CGRP. However, the increase in hindquarters flow with β-CGRP (5-70min) was similar to that seen with α-CGRP. Infusion β-CGRP was associated with renal (5-40 min) and hindquarters (5-70 min) vasodilatations like those seen with α-CGRP. However, the mesenteric vasoconstriction (5-60 min) was more rapid in offset and followed by a brief vasodilatation (80 min). Furthermore, there was no bradycardia following β-CGRP infusion, and no secondary fall in MBP.

b) Animals with bilateral common carotid probes.

Infusion of α-CGRP produced a reduction in MBP (5-70 min) associated with a tachycardia (5-70 min).

There were marked increases in common carotid flows (left, 5-70min; right, 5-70 min) associated with vasodilatations (left 5-80 min; right 5-70 min) (Fig 1). After infusion, carotid haemodynamics

returned to baseline levels, but there was a post-infusion bradycardia (100, 110 min) and secondary fall in MBP (120 min) (Fig 1), similar to the picture seen in the animals with intra-abdominal probes.

Infusion of β-CGRP produced falls in MBP (5-65 min), increases in HR (5-70 min), increases in common carotid flows (left, 5-80 min; right 5-80 min) and decreases in resistance (left, 5-80 min; right 5-70 min), similar to those seen with a α-CGRP (Figs 1 and 2). However, as with the animals bearing intra-abdominal probes, there was no bradycardia or secondary fall in MBP following infusion of β-CGRP.

These experiments show that administration of CGRP results in a controlled decrease in blood pressure while the blood supply through the left and right carotid arteries is maintained.

Example 2

Rats were infused with the following CGRP analogues at 0.6 nmol h^{-1} using the experimental procedure essentially as described in example 1. The effects on heart rate, mean arterial pressure and left and right carotid blood flow were measured essentially as described in Example 1.

CB007	ACDTATCVTH RLAGLLSRSG GMVKNNFVPT NVGSKAF
CB008	ACDTATCVTH RLAGLLSRSG GMVKSNFVPT NVGSKAF
CB009	SCDTATCVTH RLAGLLSRSG GVVKNNFVPT NVGSKAF
CB010	ACDTATCVTH RLAGLLSRSG GVVKNNFVPT NVGSEAF
CB011	ACNTATCVTH RLAGLLSRSG GMVKNNFVPT NVGSKAF

H7030 ACNTATCVTH RLAGLLSRSG GVVKSNFVPT NVGSKAF

The results are provided in Figures 3 to 13.

CGRP analogues may be synthesised using standard peptide techniques. See for example International Patent Application No. PCT/GB89/01249 filed 20th October 1989 and published European Patent Application No. EP-A-188400.

This experiment shows that administration of the CGRP analogues results in a controlled decrease in blood pressure and maintenance of blood supply through the left and right carotid arteries.

Example 3

This study investigated the tolerance and pharmacodynamics of human alpha CGRP when administered as a slow intravenous infusion to 8 healthy male volunteers. The study was designed with 3 dose levels 8 and 4 ng/kg/min and placebo infused over 3 hours. All doses were given at a rate of 0.5ml/min for 3 hours with an interval of at least 7 days between sucessive doses to each subject.

Fluid intake and output were recorded for each subject from-12-0 hours prior to dosing and from 0-24 hours following dosing.

Continuous visual monitoring of ECG was carried out during the infusions, and for at least 1 hour after the end of the infusions and 12 lead microcomputer augmented ECG recordings were made pre dose and at 1,2 and 3 hours after the start of each infusion.

SUBJECT SELECTION

A. Enrolment

Eight evaluable normal healthy male volunteers were required for this study. All participants in the study were 18-45 years of age and within 10% of their ideal weights (Metropolitan Life Insurance Company, 1983). Each subject had a medical history taken.

Within fourteen days prior to initiation of the study a complete physical examination, a 12-lead electrocardiogram, and laboratory tests of haematopoietic, hepatic and renal function, were carried out.

B. Exclusions

1. Any clinically significant deviations from the normal as evaluated by the clinical investigator, in physical examination, electrocardiography or laboratory tests.

2. Any history of drug hypersensitivity.

3. Any clinically significant allergic disease.

4. Any history of drug exposure which, in the opinion of the Principal Investigator, amounted to drug abuse.

5. No subject shall have had prior exposure to any substances which in the opinion of the Principal Investigator, would alter the safety or validity of this study.

6. Subjects who have given blood donation during the previous 3 months.

7. Subjects who after 10 minutes in the supine position, had a heart rate greater than 90 beats per minute or less than 45 beats per minute.

8. Subjects who had a supine systolic blood pressure greater than 145mm Hg or a supine diastolic pressure greater than 90mm Hg.

9. Subjects who were not echogenic.

C. Prohibitions

Subjects were instructed to abstain from alcoholic beverages and caffeine containing substances during the 24 hour period preceding and throughout the study periods. No other medication could be taken for one week prior to the study or throughout the study.

Subjects were advised not to donate blood for three months following the end of the study.

PROCEDURE

The subjects were admitted to the Institute on the morning of day -1 and remained under observation for at least 24 hours after dosing in each period. Following admission, a drug screen (including breath alcohol and urinary THC) was carried out.

Dose Administration

After an overnight fast of at least 8 hours single intravenous doses of human alpha CGRP were administered. The infusion set was then flushed with 5-10ml saline. Subjects continued to fast throughout the 3 hour infusion and for 1.5 hours after the end of the infusion.

Electrocardiography

a) Continuous visual monitoring of the ECG was carried out throughout each infusion and for at least 1 hour after the end of the infusion.

b) Twelve lead microcomputer augmented ECG recordings were made pre dose and at 1, 2 and 3 hours after the start of the infusion.

Vital Functions

Ambulatory blood pressure recordings were made every 6 minutes for 24 hours starting 30 minutes before the start of the infusions and continuing throughout the infusions and for 60 minutes after the end of the infusions, then every hour up to 12 hours, then every 2 hours up to the end of the recording period. Control measurements were made on day -1 at the corresponding times with the volunteers in the supine/erect positions similar to day 1.

Duplex Ultrasound

Ultrasound assessment of internal carotid artery, cardiac output and the middle cerebral artery (transcranial) was performed at the following time in each period:

pre dose,
10 minutes after the start of the infusion,
2 hrs 40 minutes after the start of the infusion,
10 minutes after the end of the infusion,
1 hour after the end of the infusion

The results on internal carotid volume and mean systolic blood pressure are shown in Figure 9, from which it can be seen that the blood pressure is decreased in a controlled way and that the blood supply through the internal carotid is maintained.

Figure 1

Cardiovascular variables during and after infusion of human α-CGRP (○—○) or human β-CGRP (●—●) at 0.6 nmol h^{-1}. The values show the group mean (n=8); most of the s.e.m.s are omitted, for clarity (and because the data were handled by non-parametric ANOVA). The time points at which values were significantly different from baseline are stated in the text.

Figure 2

Calculated regional vascular resistances during and after infusion of human α-CGRP (○—○) or human β-CGRP (●—●) at 0.6 nmol h^{-1}. The values show the group mean (n=8); most of the s.e.m.s are omitted, for clarity (and because the data were handled by non-parametric ANOVA). The time points at which values were significantly different from baseline are stated in the text.

Claims

1. CGRP for use in the controlled reduction of blood pressure accompanied by the maintenance of cerebrovascular blood supply.

2. A method of treatment of a human subject suffering from malignant hypertension or hypertension following cardiac surgery which comprises administering an effective amount of CGRP to the subject.

3. A pharmaceutical composition comprising CGRP for use in the treatment of malignant hypertension or hypertension following cardiac surgery.

4. A pharmaceutical composition in unit dosage form, each unit dose comprising an amount of CGRP which acts to reduce blood pressure in a controlled way whilst maintaining the cerebrovascular blood supply in combination with a pharmaceutically acceptable carrier, excipient or diluent.

5. A process for the production of a pharmaceutical composition according to claim 4 comprising bringing into association with a pharmaceutically acceptable carrier, excipient or diluent, aliquot amounts of CGRP sufficient to reduce blood pressure in a controlled way whilst maintaining the cerebrovascular blood supply to provide unit doses.

6. The use of CGRP for the manufacture of a medicament for the controlled reduction in blood pressure whilst maintaining cerebrovascular blood supply.

7. The use of CGRP for the manufacture of a medicament for the treatment of malignant hypertension or hypertension following cardiac surgery.

8. A drug for therapy of malignant hypertension or hypertension following cardiac surgery comprising a CGRP as an active ingredient.

9. A method for the treatment of malignant hypertension or hypertension following cardiac surgery which comprises administering to a patient a CGRP.

10. A controlled reducer of blood pressure and maintainer of cerebrovascular blood supply comprising CGRP.

FIG. 1

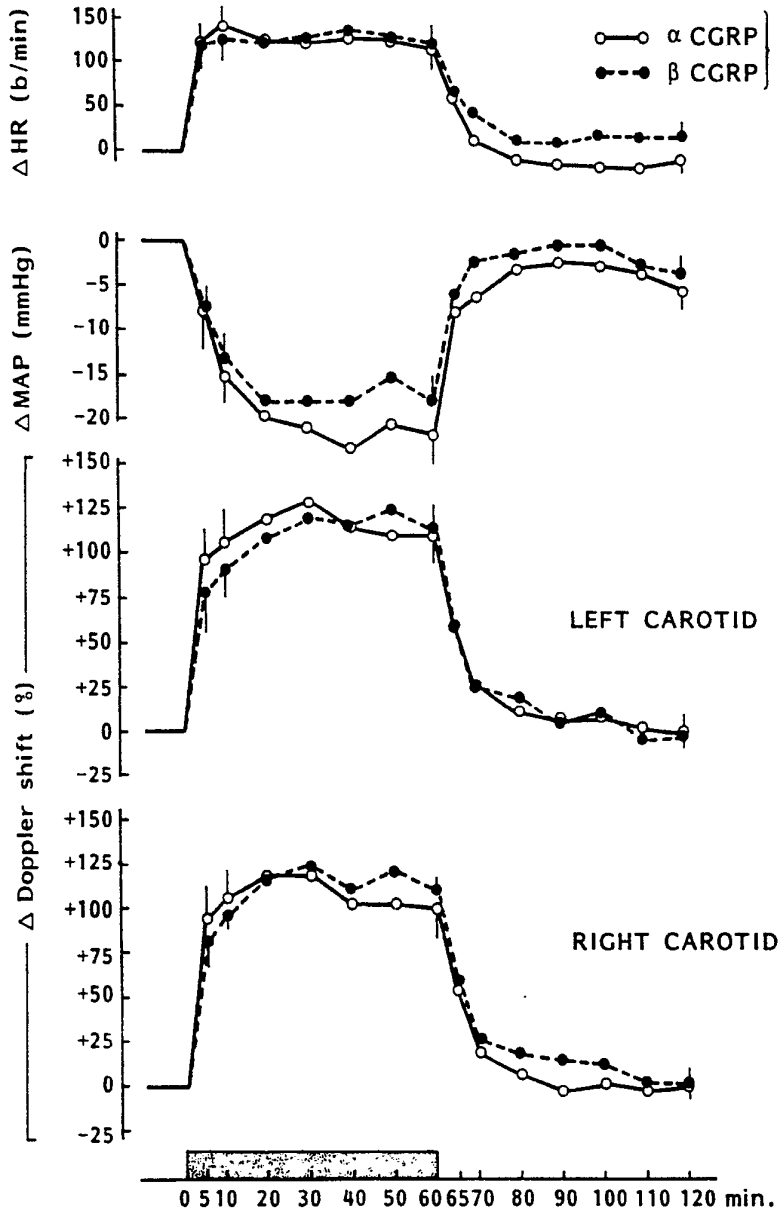

ΔHR (b/min)
150
100
50
0
α CGRP
β CGRP
0.6nmol/h
ΔMAP (mmHg)
0
-5
-10
-15
-20
Δ Doppler shift (8)
+150
+125
+100
+75
+50
+25
0
-25
LEFT CAROTID
RIGHT CAROTID
0 5 10 20 30 40 50 60 65 70 80 90 100 110 120 min.

FIG. 2

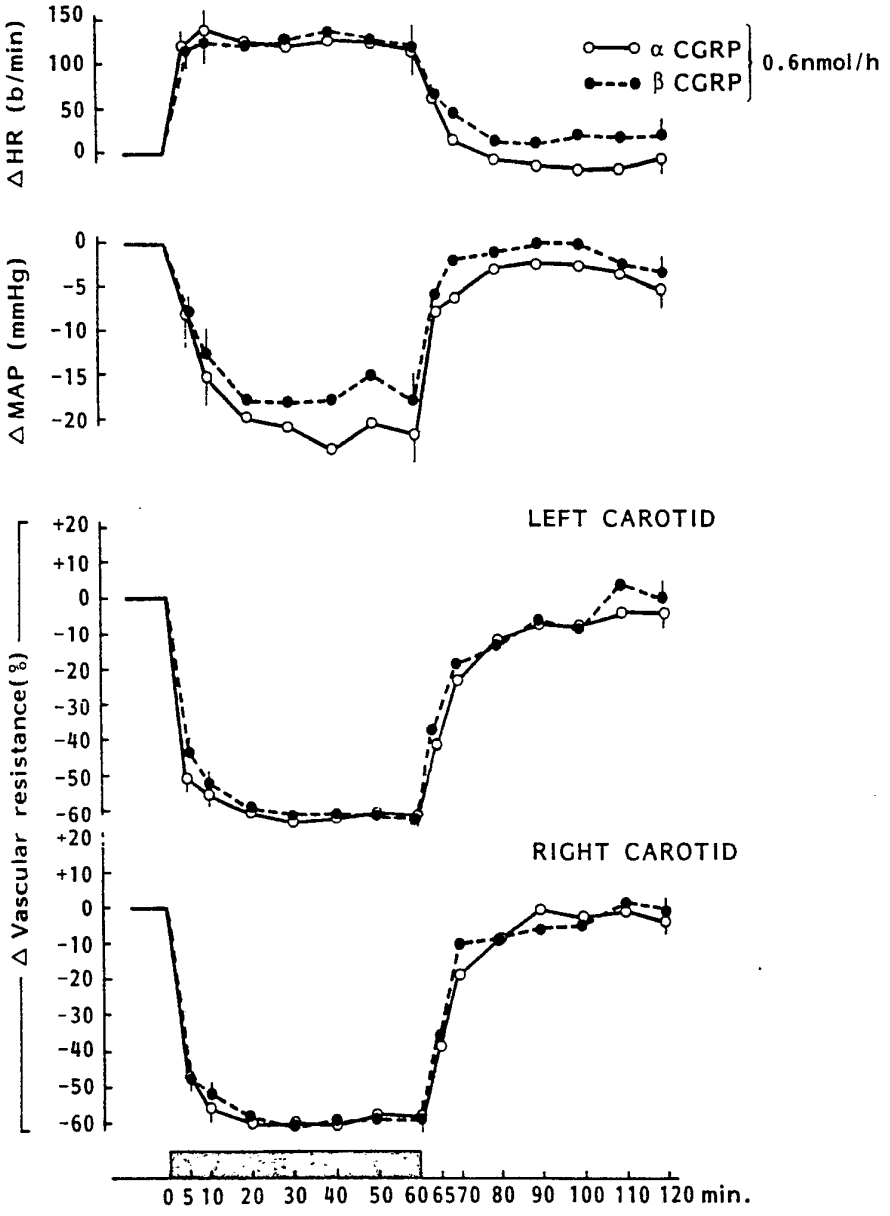

ΔHR (b/min)
150
100
50
0
α CGRP
β CGRP
0.6nmol/h
ΔMAP (mmHg)
0
-5
-10
-15
-20
LEFT CAROTID
RIGHT CAROTID
Δ Vascular resistance(%)
+20
+10
0
-10
-20
-30
-40
-50
-60
0 5 10 20 30 40 50 60 65 70 80 90 100 110 120 min.

FIG. 3

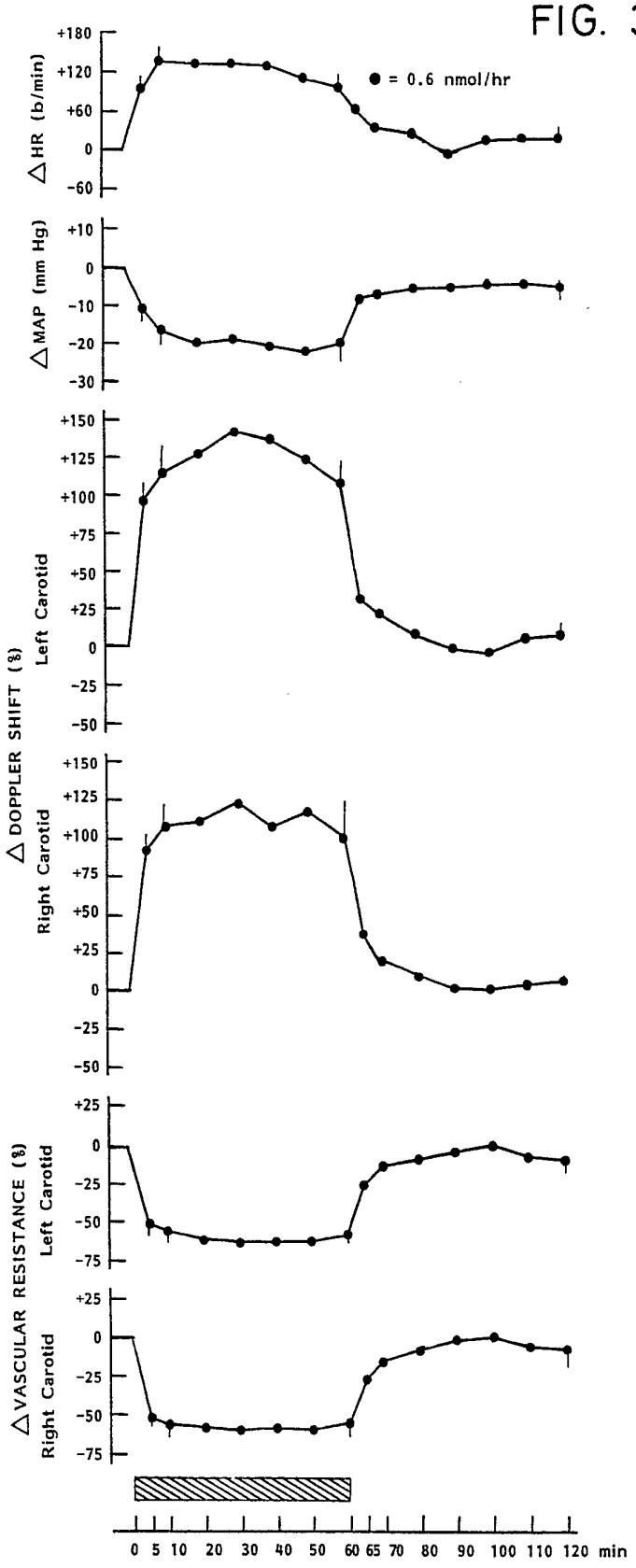

● = 0.6 nmol/hr
△HR (b/min)
+180
+120
+60
0
-60
△MAP (mm Hg)
+10
0
-10
-20
-30
△DOPPLER SHIFT (%)
Left Carotid
+150
+125
+100
+75
+50
+25
0
-25
-50
Right Carotid
△VASCULAR RESISTANCE (%)
Left Carotid
+25
0
-25
-50
-75
Right Carotid
0 5 10 20 30 40 50 60 65 70 80 90 100 110 120 min

FIG. 4

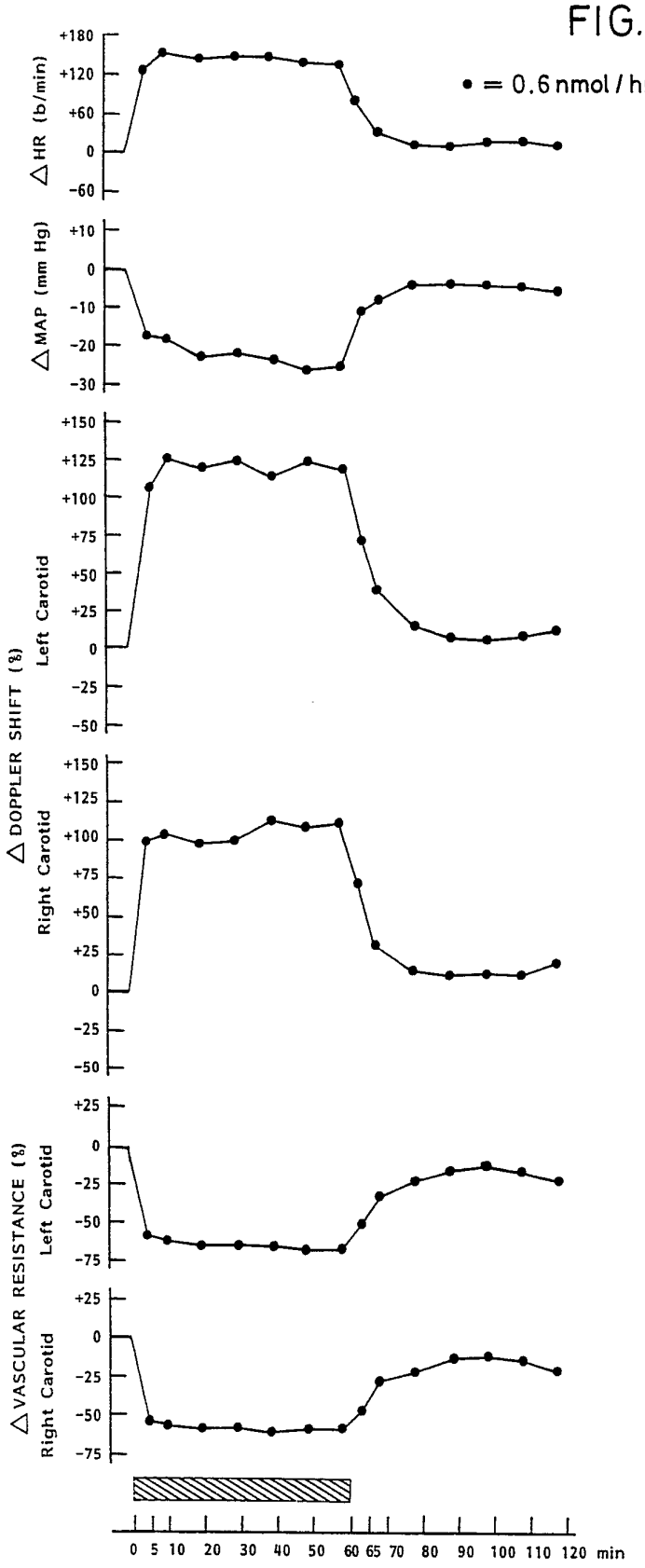

• = 0.6 nmol / hr
△ HR (b/min)
+180
+120
+60
0
-60
△ MAP (mm Hg)
+10
0
-10
-20
-30
△ DOPPLER SHIFT (%)
Left Carotid
+150
+125
+100
+75
+50
+25
0
-25
-50
Right Carotid
△ VASCULAR RESISTANCE (%)
Left Carotid
+25
0
-25
-50
-75
Right Carotid
0 5 10 20 30 40 50 60 65 70 80 90 100 110 120 min

FIG. 5

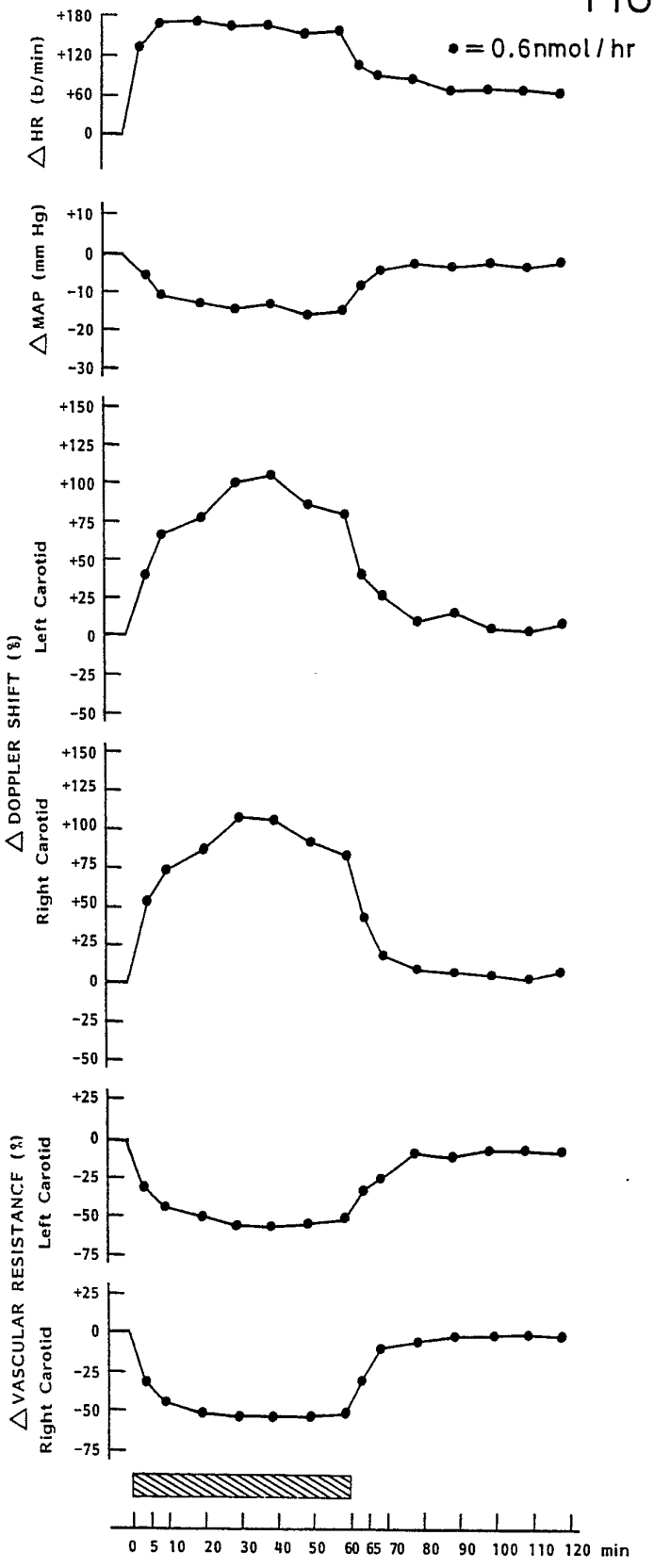

• = 0.6nmol / hr
△HR (b/min)
+180
+120
+60
0
△MAP (mm Hg)
+10
0
-10
-20
-30
△DOPPLER SHIFT (%)
Left Carotid
+150
+125
+100
+75
+50
+25
0
-25
-50
Right Carotid
△VASCULAR RESISTANCE (%)
Left Carotid
+25
0
-25
-50
-75
Right Carotid
0 5 10 20 30 40 50 60 65 70 80 90 100 110 120 min

FIG. 6

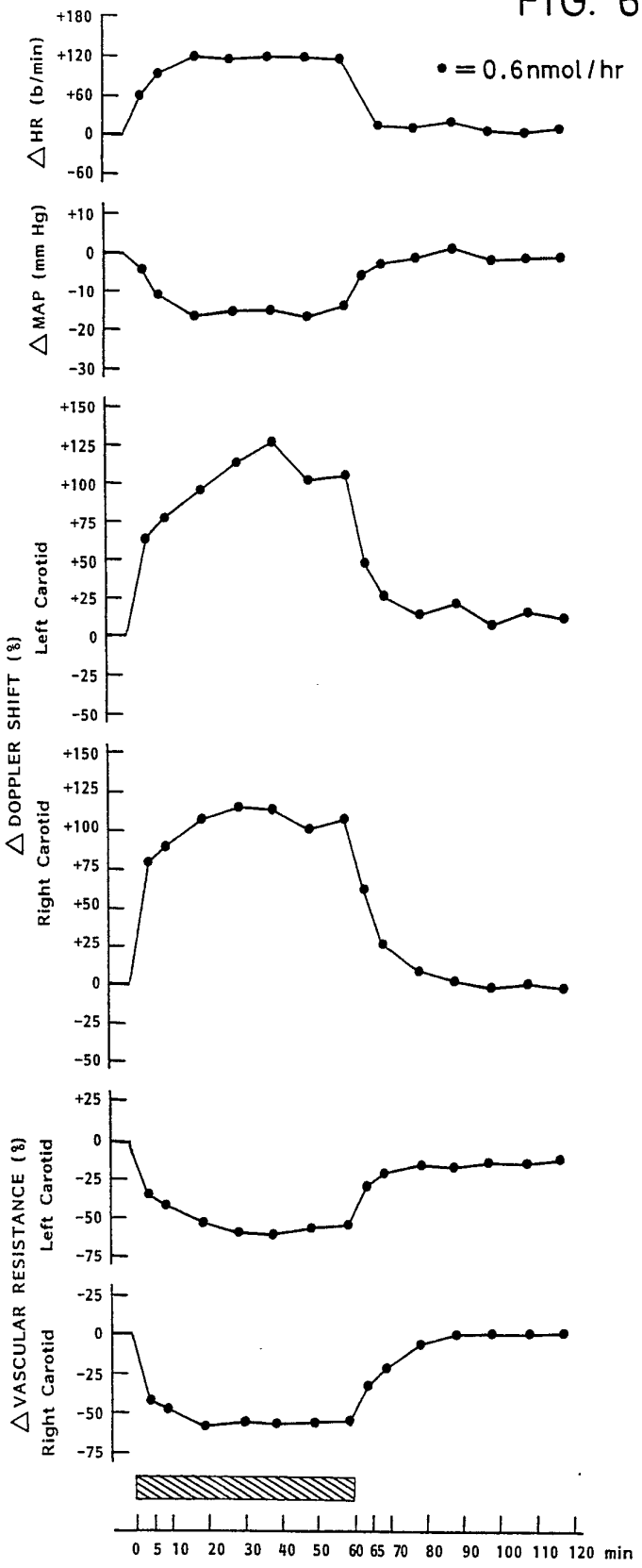

• = 0.6nmol/hr
△HR (b/min)
+180
+120
+60
0
-60
△MAP (mm Hg)
+10
0
-10
-20
-30
△DOPPLER SHIFT (%)
Left Carotid
+150
+125
+100
+75
+50
+25
0
-25
-50
Right Carotid
△VASCULAR RESISTANCE (%)
Left Carotid
+25
0
-25
-50
-75
Right Carotid
0 5 10 20 30 40 50 60 65 70 80 90 100 110 120 min

FIG. 7

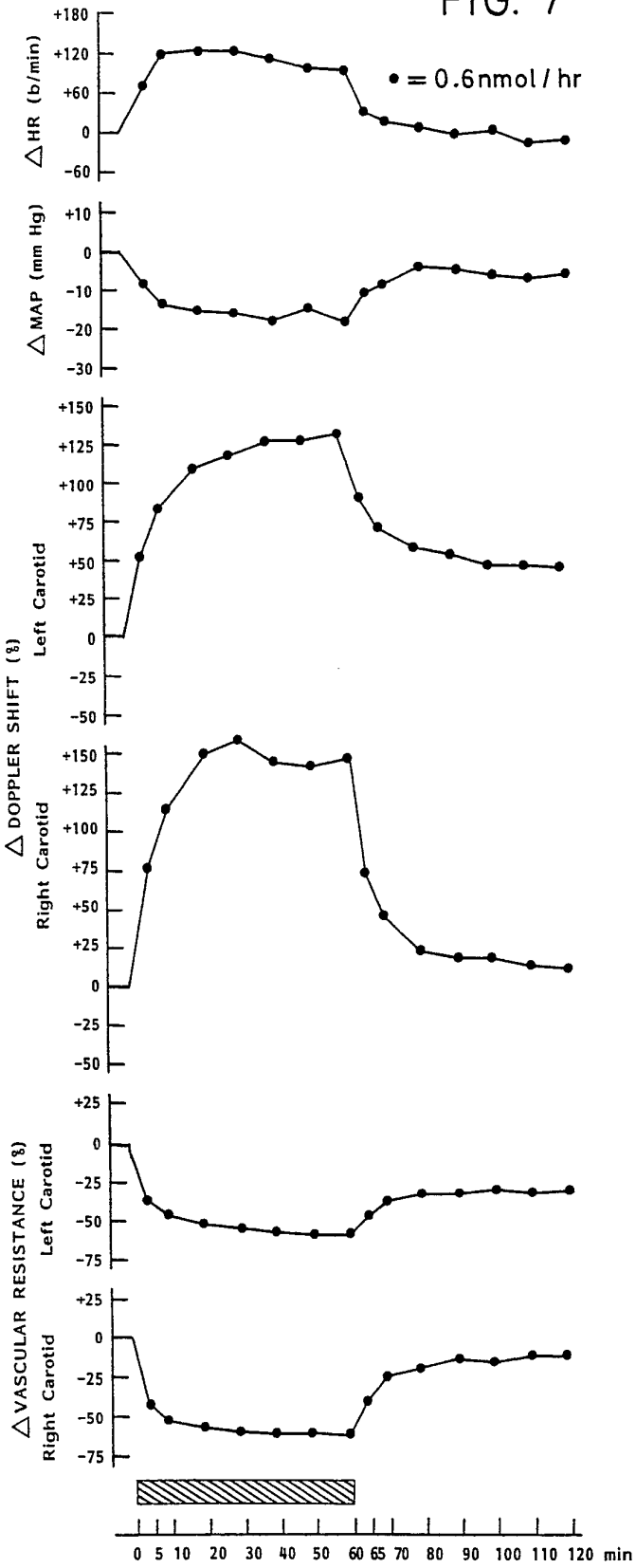

• = 0.6nmol / hr
△HR (b/min)
+180
+120
+60
0
-60
△MAP (mm Hg)
+10
0
-10
-20
-30
△DOPPLER SHIFT (%)
Left Carotid
+150
+125
+100
+75
+50
+25
0
-25
-50
Right Carotid
△VASCULAR RESISTANCE (%)
Left Carotid
+25
0
-25
-50
-75
Right Carotid
0 5 10 20 30 40 50 60 65 70 80 90 100 110 120 min

FIG. 8

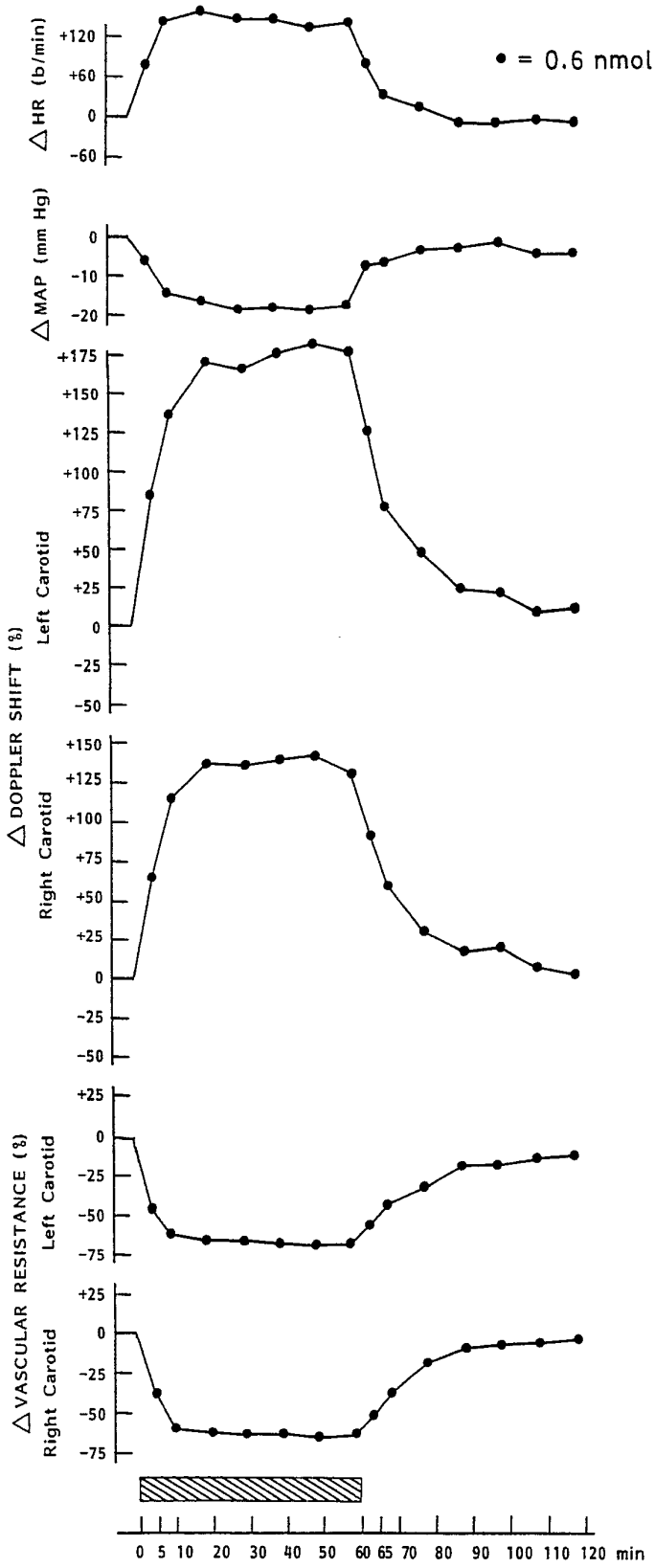
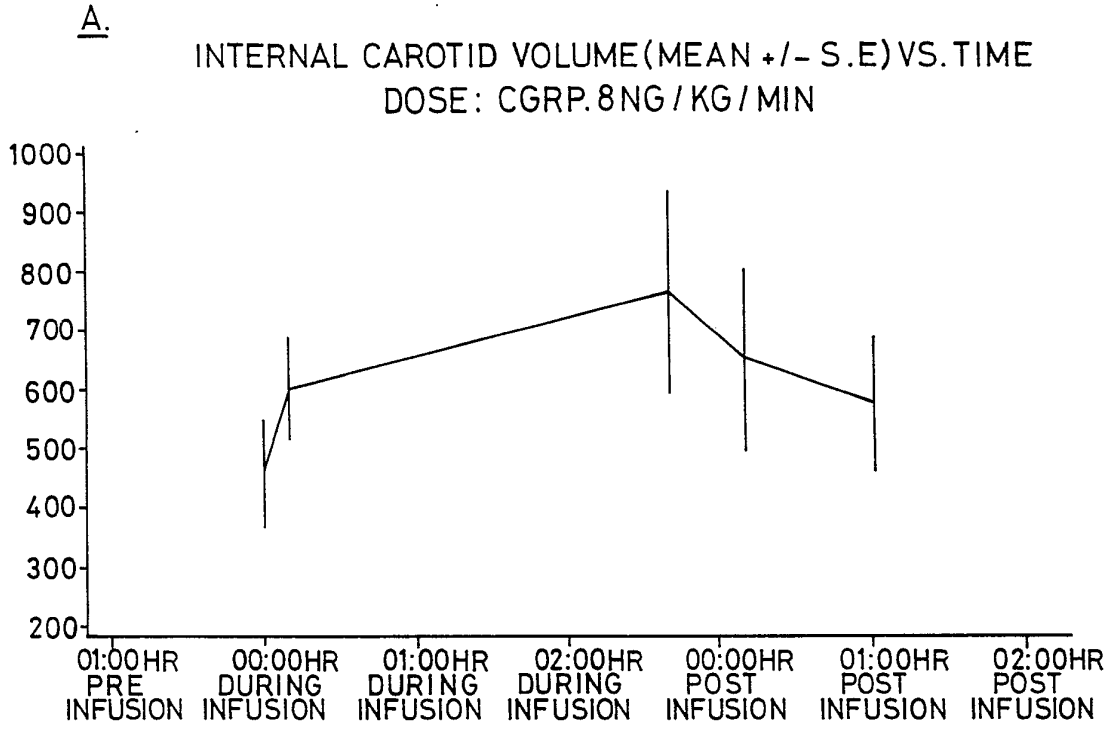
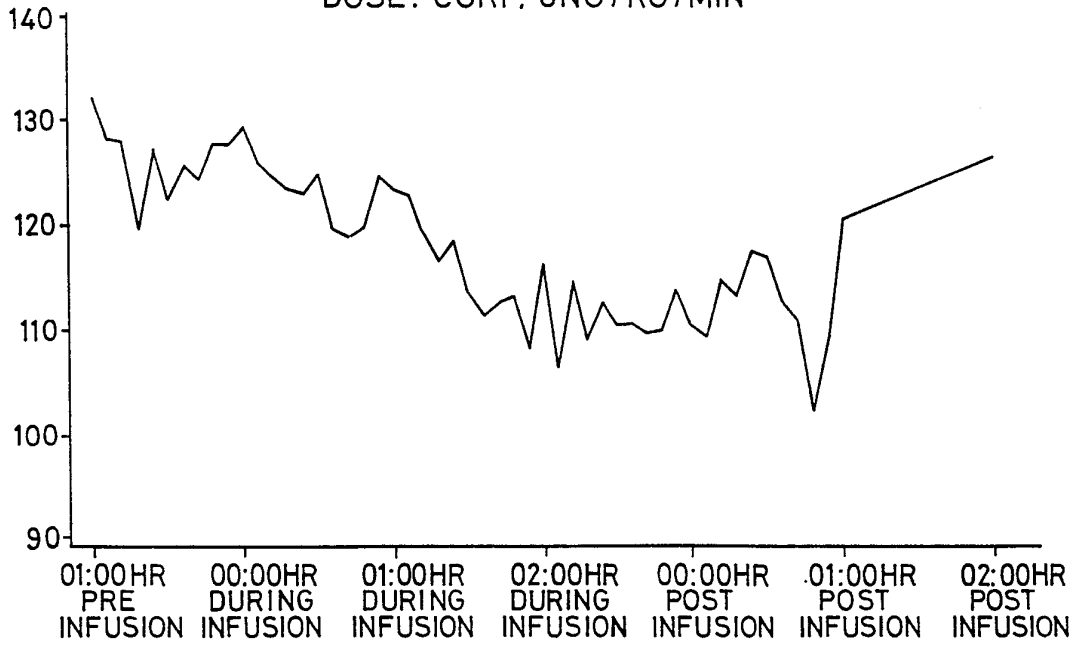

• = 0.6 nmol / hr
△HR (b/min)
+120
+60
0
-60
△MAP (mm Hg)
0
-10
-20
△DOPPLER SHIFT (8)
Left Carotid
+175
+150
+125
+100
+75
+50
+25
0
-25
-50
Right Carotid
+150
+125
+100
+75
+50
+25
0
-25
-50
△VASCULAR RESISTANCE (8)
Left Carotid
+25
0
-25
-50
-75
Right Carotid
+25
0
-25
-50
-75
0 5 10 20 30 40 50 60 65 70 80 90 100 110 120 min

FIG. 9

A.


INTERNAL CAROTID VOLUME (MEAN +/- S.E) VS. TIME
DOSE: CGRP. 8NG / KG / MIN
1000
900
800
700
600
500
400
300
200
01:00HR PRE INFUSION
00:00HR DURING INFUSION
01:00HR DURING INFUSION
02:00HR DURING INFUSION
00:00HR POST INFUSION
01:00HR POST INFUSION
02:00HR POST INFUSION

B.


MEAN SYSTOLIC BLOOD PRESSURE VS. TIME
DOSE: CGRP. 8NG / KG / MIN
140
130
120
110
100
90
01:00HR PRE INFUSION
00:00HR DURING INFUSION
01:00HR DURING INFUSION
02:00HR DURING INFUSION
00:00HR POST INFUSION
01:00HR POST INFUSION
02:00HR POST INFUSION

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 90/00594

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: C 07 K 99/46, C 07 K 7/10, A 61 K 37/02

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	C 07 K, A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	American Society for Clinical Pharmacology and Therapeutics, volume 45, no. 2, February 1989, P. Salmon et al.: "A single rising intravenous dose tolerance and pharmacodynamic study of human calcitonin gene related peptide (CGRP) in healthy male volunteers", page 17 see the whole article	1,3-8,10
Y	Regulatory Peptides, volume 20, no. 3, 1988, Elsevier Science Publishers BV, L. Edvinsson et al.: "Neurokinin A in cerebral vessels: characterization, localization and effects in vitro", pages 181-197 see the whole article	1,3-8,10
Y	Stroke, volume 20, no. 1, 1989,	1,3-8,10

./.

* Special categories of cited documents: [10]

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

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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12th July 1990	10.08.90
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	H. Daniels H. DANIELS

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages	Relevant to Claim No.
	K. Hongo MD, et al.: "Effect on subarachnoid hemorrhage on calcitonin gene-related peptide-induced relaxation in rabbit basilar artery", pages 100-104 see the whole article	
	--	
Y	Journal of Cerebral Blood Flow and Metabolism, volume 7, no. 6, December 1987, Raven Press Ltd., (New York, US), L. Edvinsson et al.: "Calcitonin gene-related peptide and cerebral blood vessels: Distribution and vasomotor effects", pages 720-728 see the whole article	1,3-8,10
	--	
A	The Lancet, 6 July 1985, S.I. Girgis et al.: "Calcitonin gene-related peptide: Potent vasodilator and major product of calcitonin gene", pages 14-16 see the whole article	

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 2, 9, because they relate to subject matter not required to be searched by this Authority, namely:

See PCT-Rule 39.1(iv)
Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.