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(54) Title: USE OF AN AMINO-TETRALINE DERIVATIVE AS ANTI-ARRHYTHMIC AGENT

(57) Abstract: A method of treatment of cardiac arrhythmias comprising the administration of an effective amount of (+/-)-(R,S)-5,6-dihydroxy-2-methylamino-1,2,3,4-tetrahydro-naphthalene hydrochloride (CHF-1024) to a patient in need thereof.



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USE OF AN AMINO-TETRALINE DERIVATIVE AS ANTI-ARRHYTHMIC AGENT

The present invention relates to a method of treatment of cardiac arrhythmias comprising the administration of an effective amount of (+/-)-(R,S)-5,6-dihydroxy-2-methylamino-1,2,3,4-tetrahydro-naphthalene hydrochloride (CHF-1024) to a patient in need thereof.

5 Background of the invention

Regional differences in action potential characteristics in the normal ventricular myocardium, both transmurally as well as in the base to apex direction, has been extensively described in several animal species and in the human heart (Watanabe et al. 1983; Shipsey et al. 1997; Li et al. 1998; Volk et al. 1999, Stankovicova et al. 2000; Antzelevitch & Fish, 2001; Burton & Cobbe, 2001). Spatial dispersion of action potential duration (APD) is physiological and plays an important role in the maintenance of normal ventricular recovery gradient and electrical stability of ventricular myocardium. In the rat left ventricle, a continuous transmural gradient of APD
10 al. 1999, Stankovicova et al. 2000; Antzelevitch & Fish, 2001; Burton & Cobbe, 2001). Spatial dispersion of action potential duration (APD) is physiological and plays an important role in the maintenance of normal ventricular recovery gradient and electrical stability of ventricular myocardium. In the rat left ventricle, a continuous transmural gradient of APD
15 has been described and mainly attributed to inhomogeneities in the distribution of the transient outward K^+ current (I_{to}) (Li et al. 1998; Volk et al. 1999). Sub-endocardial cells exhibit action potentials significantly longer than sub-epicardial cells, while APD recorded from the mid-myocardial regions attained intermediate values (Volk et al. 1999).

20 Changes in basic dispersion in the recovery of excitability in the heart have long been associated with the occurrence of ventricular arrhythmias, such as ventricular ectopy, ventricular tachycardia, and ventricular fibrillation. Reentrant excitation and triggered activity from early (EAD) and delayed after depolarizations (DAD) are recognised as common mechanisms underlying
25 ventricular tachyarrhythmias due to altered dispersion of repolarization

(Amlie, 1997; Pastore & Rosenbaum 2000; Antzelevitch & Fish, 2001; Yan et al. 2001a; Akar et al. 2002; Nerbonne & Guo, 2002; Akar & Rosenbaum 2003; Obreztkhikova et al. 2003; Stilli et al. 2004).

Cardiac hypertrophy induces an average prolongation of APD that affects repolarization heterogeneity (Gomez et al., 1997; Shipsey et al. 1997; Volk et al. 2001; Yan et al. 2001b; Yang et al. 2002). The electrical remodeling of the hypertrophied heart leads to a myocardial substrate predisposed to arrhythmia development, particularly under specific conditions affecting APD and APD heterogeneity, such as sympathetic stimulation (Stilli D. et al 2001). Actually, studies performed on isolated normal ventricular myocytes have shown that adrenergic stimulation results in a dose-dependent prolongation of APDs and induces EADs and DADs, suggesting a possible pathway for arrhythmogenesis following adrenergic stimulation in the intact heart (Priori & Corr, 1990).

Neurohormonal activation concurs to the patho-physiology of cardiac pressure overload, in addition to elevation of blood pressure. This activation is one of the hallmarks of chronic heart failure and is strongly related to the prognosis (Tjeerdsma et al. 2001). (+/-)-(R,S)-5,6-dihydroxy-2-methylamino-1,2,3,4-tetrahydro-naphthalene hydrochloride (CHF-1024), an α 2-adrenergic/DA2-dopaminergic agonist, has been proposed as additional treatment in cardiac hypertrophy and failure due to its well documented inhibitory effect on catecholamine release (Masson et al. 2001, Tjeerdsma et al. 2001, Rossoni et al. 2003). Indeed, it has been proved that CHF-1024 reduces cardiac adrenergic drive and systemic hypertension, and limits left ventricular perivascular collagen deposition in the hypertrophic heart, in a dose-dependent manner.

It has now been found that CHF-1024 is also effective in preventing the occurrence of arrhythmias that often favour the transition from compensated

hypertrophy towards cardiac insufficiency and increase the incidence of morbidity and mortality in the hypertrophic disease. The antiarrhythmic action of CHF-1024 was evaluated by measuring 1) the effects of CHF-1024 on cardiac electrical instability of the hypertrophied heart and 2) possible mechanisms underlying these effects, at the cellular level, in a rat model of pressure-overload ventricular hypertrophy induced by abdominal aortic coarctation.

METHODS

Study population and experimental protocol

10 The study population consisted of 71 6-mo-old male Wistar rats (*Rattus Norvegicus*). All animals were housed in groups of five individuals, from weaning (1 month after birth) until the onset of the experiments (age of 5 months), in clear Plexiglas cages measuring 55 x 35 x 20 cm and kept in a temperature-controlled room at 20-24°C, with the light on between 7 AM and 15 7 PM. The bedding of the cages consisted of wood shavings, and food and water were freely available.

 All animals were chronically instrumented with a miniaturized transmitter for telemetry ECG recording. In 57 rats, abdominal aortic coarctation was performed during transmitter implantation in order to induce left ventricular hypertrophy (AC group), while the remaining animals were used as control group (C group). During surgery, in AC rats, an osmotic pump was also implanted subcutaneously, in the interscapular region, for continuous infusion of CHF-1024 at four different concentrations. Two high concentrations of CHF-1024 were used (6mg/Kg/die, n. of rats = 7, AC_{D3} group; 2mg/Kg/die, n. of rats = 10, AC_{D2} group), according to Masson et al. 20 (2001), who studied the effect of this treatment in a similar rat model of cardiac pressure-overload. Additional lower concentrations of CHF-1024 were tested, respectively equal to 0.67mg/Kg/die (n. of rats = 10, AC_{D1} group) and 25

0.067mg/Kg/die (n. of rats = 12, AC_{D0} group). Eighteen rats were used as control animals (vehicle infusion, AC₋ group).

One month after surgery, long-lasting telemetry ECG recordings were performed in baseline conditions and during autonomic activation obtained by exposing the animals to an acute social stress episode (see below), in order to evaluate arrhythmia vulnerability. Part of the animals were provided with a silastic heart cannula through the jugular vein for blood sampling in conscious, freely moving rats. Blood samples were used for determining plasma levels of catecholamines (5 AC₋, 10 AC_{D0} and 5 C) and CHF-1024 (most of AC animals). Then, the animals were sacrificed and the hearts used for electrophysiological analyses on isolated ventricular myocytes, by means of the patch clamp technique.

During all surgical procedures, the animals were anesthetized with droperidol plus fentanyl citrate (Leptofen, Pharmacia & Upjohn, Milan, Italy; 0.1 ml/100 g, i.m.) and treated with antibiotic therapy with gentamicine (Aagent, Fatro Milan, Italy, 0.2 ml/Kg i.m.) for the subsequent 3 days.

Implant of ECG transmitter and osmotic pump, and coarctation of the abdominal aorta

In accordance with a previously described procedure (Sgoifo et al. 1996), the body of the ECG transmitter (models TA11CTA-F40, Data Sciences, St. Paul, MN, USA) was placed in the abdominal cavity, one lead was fixed on the dorsal surface of the xiphoid process and the other wire was subcutaneously tunneled towards the anterior mediastinum and sutured close to the right atrium .

In 57 animals, abdominal aortic coarctation was also performed (De Chastonay et al. 1983). Briefly, the aorta between the two renal arteries was dissected free and a 0.7-mm-diameter blunted needle was laid alongside the exposed segment of the vessel. A silk ligature was passed under the aorta and

the needle, and tied securely between the two renal arteries . The needle was carefully withdrawn and the abdominal wall was sutured. This procedure led to about 55% reduction in the lumen of the aorta. An osmotic pump (ALZET, Cupertino, CA, USA) was placed subcutaneously in the interscapular region
5 for chronic administration of CHF-1024 or vehicle.

Cannula implant

Three weeks after surgery, most animals were anesthetized and provided with a silastic heart cannula (diameter 0.9 mm, internal diameter 0.5 mm; Dow Corning, Midland, MI) through the right jugular vein, with one end
10 reaching the entrance of the right atrium and the other one externalized on the top of the skull . This method allows frequent blood sampling in conscious, undisturbed and freely moving rats. The animals were then individually housed in Plexiglas cylindrical cages (diameter 40 cm, height 60 cm) and were allowed to recover for 7 days before the onset of experiments. The second and
15 the fourth day after surgery, each rat was connected to a blood sampling polyethylene tubing (length 70 cm, diameter 1.4 mm, internal diameter 0.7 mm) to test the functionality of the silastic heart cannula and to habituate the animal to the sampling procedure. On the day of the experiment the polyethylene tubing was connected 60 min before the blood sample
20 withdrawal.

To test whether CHF-1024 at low concentration (0,067mg/Kg/die) reduces plasma catecholamine levels, as already shown for higher concentrations, blood samples (2 ml) were withdrawn from 5 AC₋, 10 AC_{D0} and 5 C rats, in baseline conditions and during the exposure to the social stress
25 episode.

After each blood sample withdrawal, the same amount of heparinized physiological solution was transfused trough the catheter to avoid changes in hemodynamics.

In addition, a blood sample was taken from all the instrumented animals before sacrifice, to evaluate plasma concentration of CHF-1024 .

Social stress and ECG data acquisition and processing

The social stress procedure consisted of introducing the instrumented
5 animal (intruder) in the territory of a conspecific male (resident) belonging to
an aggressive wild strain of rats (*Rattus Norvegicus*, Wild Type Groningen,
WTG) (resident-intruder test; Martinez et al. 1998). It is known that this
procedure induces an intense autonomic stimulation associated with a shift of
the simphathovagal balance towards a sympathetic predominance (Sgoifo et
10 al. 1999) .

The telemetry ECG was continuously recorded throughout the
experimental session consisting of two 15-min periods, during which the
animal was first left alone and undisturbed in its home cage (baseline period),
then exposed to the stress procedure. The ECG signals were collected by a
15 receiver (model CTR85-SA, Data Sciences, St. Paul, MN, USA) placed under
the experimental cages. The signals were monitored on an oscilloscope and
simultaneously routed to a personal computer, via an analog-to-digital
conversion board (12 bits, 1.000 Hz sampling rate), for permanent storage . A
software package developed in our laboratory was used for interactively
20 analyzing ECG data in order to determine the mean R-R interval and the
incidence of ventricular arrhythmic events, in each experimental condition.

Cellular Electrophysiological studies

Cell isolation. After ECG recording and blood sampling (see above)
each rat was sacrificed and single left ventricular myocytes were enzymatically
25 isolated as previously described (Zaniboni et al. 2000). Briefly, the rat heart
was removed and rapidly perfused at 37°C by means of an aortic cannula with
the following sequence of solutions (see below): (i) a calcium free solution for
5 minutes to remove the blood, (ii) a low calcium solution (0.1 mM) plus 1

mg/ml type 2 collagenase (Worthington Biochemical Corporation), and 0.1 mg/ml type XIV protease (Sigma, Milan, Italy), for about 20 min, and (iii) an enzyme free low calcium solution for 5 min. The left ventricle was then minced and shaken for 10 min in the low-calcium solution. Myocytes were stored at room temperature in the control solution. All experiments were performed within 8 h after isolation. All myocytes used in this study had well-defined striations and did not spontaneously contract.

Electrophysiological methods and data analysis. Suction pipettes were made from borosilicate capillary tubing (Harvard Apparatus LTD, Edenbridge, UK) with an access resistance of 2-4 M Ω when filled. Single myocytes were brought in whole-cell configuration and electrically paced in current-clamp mode using an Axoclamp 2B amplifier (Axon Instruments, Foster City, CA). Data recordings and analysis were performed via the pCLAMP6 software (Axon Instruments, Foster City, CA). Transmembrane potential (V_m) was measured in whole-cell configuration using an Axoclamp 2B amplifier (Axon Instruments, Foster City, CA). Membrane capacitance (C_m) was measured by intracellular injection of subthreshold hyperpolarizing constant current pulses (100 ms duration) (Huelsing et al. 1999). Action potentials were elicited by injection of brief (3 ms) depolarizing constant current pulses (~50% above current threshold) via a reversible electrode inside the suction pipette at the pacing rate of 1 Hz and sampled at 5 kHz. Time was allowed for action potentials to reach a steady state configuration (usually within 20 beats). Then, 10 consecutive action potentials were recorded and averaged, and the following parameters were measured: resting potential (V_r) and action potential duration at -20 and -50 mV (APD_{20} and APD_{50}) (Figure 5 c). APD_{20} and APD_{50} were measured as the distance between the time of the peak of the first order derivative of the initial rapid depolarization (taken as an index of maximal activation of I_{Na} current) and the time, during repolarization, when

membrane potential reached respectively -20 and -50 mV.

Solutions. The calcium-free solution contained the following (in mM): 126 NaCl, 22 dextrose, 5.0 MgCl_2 , 4.4 KCl, 20 taurine, 5 creatine, 5 Na pyruvate, 1 NaH_2PO_4 , and 24 HEPES (pH = 7.4, adjusted with NaOH). The solution was gassed with 100% O_2 . Control solution for cell bathing during experiments contained the following (in mM): 126 NaCl, 11 dextrose, 5.4 KCl, 1.0 MgCl_2 , 1.08 CaCl_2 and 24 HEPES (pH = 7.4, adjusted with NaOH). Normal pipette filling solution contained the following (in mM): 113 KCl, 10 NaCl, 5.5 dextrose, 5 K_2ATP , 0.5 MgCl_2 and 10 HEPES (pH = 7.1 adjusted with KOH). The temperature of the solutions in the cell bath was $36 \pm 0.2^\circ\text{C}$.

Statistical analysis

Normal distribution of variables was checked by means of the Kolmogorov-Smirnov test. Statistics of variables normally distributed (all variables except the number of ventricular arrhythmias during baseline) included: means $\pm$ standard error (SEM), unpaired Student's t-test, one-way analysis of variance (post-hoc analysis: Games-Howell test). Non-parametric statistical analysis was used (Mann-Witney U-test) to compare data relating to the incidence of arrhythmias during baseline and social stress period (SPSS statistical package: SPSS, Chicago, IL). Statistical significance was set at $p < 0.05$.

RESULTS

Telemetry ECG data

In all groups of animals, the average values of R-R interval were approximately equal to 200 ms in baseline conditions and significantly decreased during social stress exposure (approximately -30%, $p < 0.01$) (Figure 1). No significant differences among groups were observed relative to the behaviour of R-R interval, however a dose-dependent trend towards a reduction in the heart rate response to stress exposure was found in animals

treated with CHF-1024 (Figure 1).

In all animals, ventricular arrhythmic events (VAEs), constituted by isolated premature beats, were negligible during baseline while they markedly increased during social challenge ($p < 0.05$). Although the electrocardiographic response to stress exposure was qualitatively similar in all animals, statistically significant differences among groups were observed during the social challenge, when the incidence of VAEs was significantly higher in AC_ rats, without pharmacological treatment, as compared to control animals (VAEs: 12 ± 2 in AC_ vs. 5 ± 1 in C; $p < 0.01$) (Figure 2). The higher vulnerability to stress induced arrhythmias associated with the presence of cardiac hypertrophy was completely abolished by CHF-1024 administration, independently of the concentration used (Figure 2).

Analysis of blood samples

CHF-1024. The performance of the osmotic pump in releasing CHF-1024 throughout the trial (one month) was tested by analyzing plasma levels of the drug in the blood samples withdrawn from AC animals, before sacrifice. A clear dose dependency was found in CHF-1024 plasma concentration, as shown in Table 1.

Table 1.

CHF 1024 R (+)			CHF 1024 S (-)		
groups	ng/ml	pmol/ml	groups	ng/ml	pmol/ml
AC _{D0}	0.230±0.06	1.188±0.31	AC _{D0}	0.441±0.08	2.283±0.44
AC _{D1}	1.168±0.16	6.045±0.81	AC _{D1}	2.411±0.36	12.47±1.86
AC _{D2}	2.177±0.32	11.268±1.64	AC _{D2}	6.821±0.46	32.298±2.38
AC _{D3}	5.273±1.51	27.288±7.79	AC _{D3}	15.063±3.09	77.949±16.0

Plasma Norepinephrine. C and AC₋ animals showed comparable average values of norepinephrine (NE) plasma levels in baseline conditions (range: 170 - 549 pg/ml) (Table 2). As expected, the exposure to social stress significantly increased plasma NE concentration (more than three times), in both groups. Although a similar trend was observed in AC_{D0} rats, the administration of CHF-1024 produced an evident reduction (approximately 50%) of NE plasma levels in the two experimental conditions (Table 2). These data are in accordance with those reported in previous studies where higher doses of CHF-1024 were used (Masson et al. 2001; Rossoni et al. 2003), however we demonstrated that catecholamine release can be reduced even by administration of CHF-1024 at very low concentration (0.067mg/Kg/die, AC_{D0} group) (Table 2).

Table 2

NE plasma level (pg/ml)		
groups	baseline	Stress
AC _{D0}	149±64	589±107
C+AC ₋	313±119	1040±190

$p=0.1$ * $p<0.05$

Cellular electrophysiological study

Membrane capacitance (Cm), usually taken as an index of cell size, was found significantly increased, as expected, in cells isolated from banded non-treated animals (AC₋) compared with normal animals (C). The treatment with CHF-1024 at the lowest concentration prevented the increase of Cm, blunting the hypertrophic effect of aortic banding. On the contrary, cells isolated from animals treated with CHF-1024 at the three higher doses showed a significant increase in this parameter, also in comparison with cells isolated from AC₋

(Table 3 and Figure 3).

Table 3. Mean values $\pm$ SEM of Cm.

groups	Cm (pF)
C	171.5 $\pm$ 6.7
AC	196.2 $\pm$ 8.2
AC _{D0}	177.9 $\pm$ 7.4
AC _{D1}	266.5 $\pm$ 11.7
AC _{D2}	259.9 $\pm$ 16.1
AC _{D3}	260.3 $\pm$ 15.7

- 5 APD₂₀ and APD₅₀ measured at 1 Hz were significantly higher in cells isolated from AC_ animals compared with C group, most likely as a consequence of an hypertrophy-induced decrease in I_{to} current density in early phase of repolarization (Bryant et al. 1999), and increase in Na-Ca exchanger depolarizing activity, in the later phase (Gomez et al. 2002). In the late phase
- 10 of repolarization (-50 mV) the treatment with CHF-1024 at all the 4 doses prevented the hypertrophy-induced increase in APD (Table 4 and Figures 4-5).

Table 4. Mean values $\pm$ SEM of APD₂₀ and APD₅₀.

groups	APD20 (ms)	APD50 (ms)
C	25.2 $\pm$ 1.7	90.2 $\pm$ 4
AC	34.1 $\pm$ 3	104.9 $\pm$ 5.4
AC _{D0}	39.5 $\pm$ 4.3	89 $\pm$ 6.9
AC _{D1}	29.2 $\pm$ 4.4	72.8 $\pm$ 8.6
AC _{D2}	24.4 $\pm$ 4	73.7 $\pm$ 9.2
AC _{D3}	36.3 $\pm$ 4.3	82.1 $\pm$ 13

Morphometric data obtained in additional animals belonging to C, AC_

and AC_{D1} groups show that, unlike Cm, cell diameters are comarable in AC_{D1} and AC_ animals (Figure 6).

The above data show that CHF-1024 is indeed antiarrhythmic since it reduces significantly the incidence of stress induced ventricular arrhythmias in
5 the model of pressure-overload cardiac hypertrophy under study causing a partial recovery of cellular repolarization properties to a normal phenotype, even at very low doses.

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CLAIMS

1. A method of treatment of cardiac arrhythmias comprising the administration of an effective amount of (+/-)-(R,S)-5,6-dihydroxy-2-
5 methylamino-1,2,3,4-tetrahydro-naphthalene hydrochloride (CHF-1024) to a patient in need thereof.
2. A method according to claim 1 wherein the patient is affected by heart hypertrophy.

R-R interval

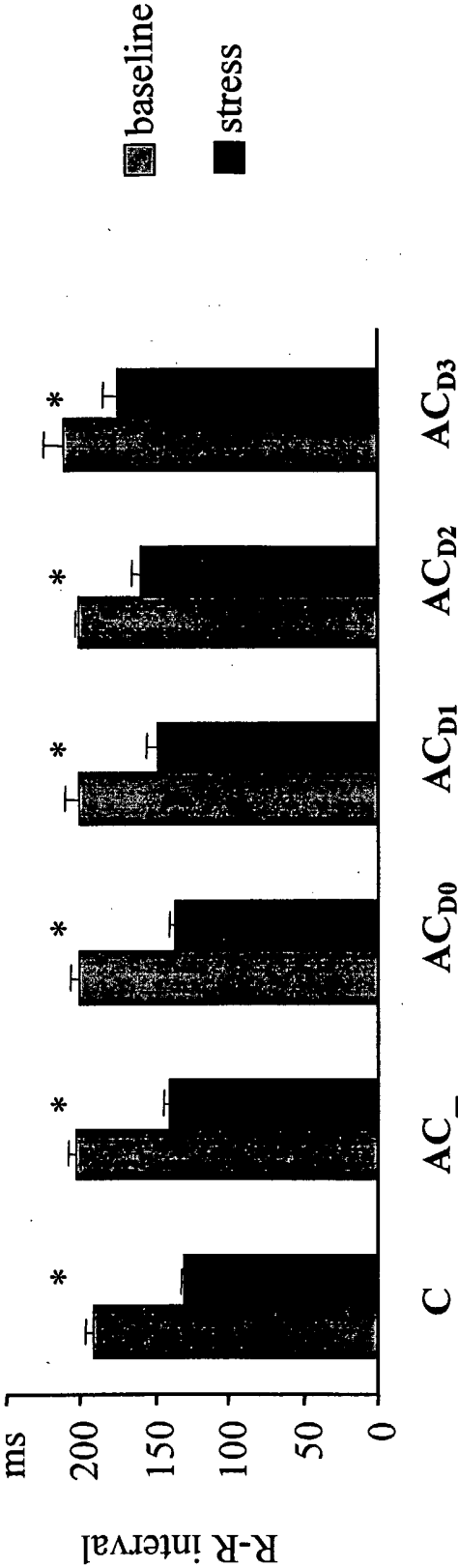


Figure 1: R-R interval (mean $\pm$ SEM) during baseline and stress periods, in the experimental groups.

* significant differences between baseline and stress period, in each group

Ventricular arrhythmic events (VAEs)

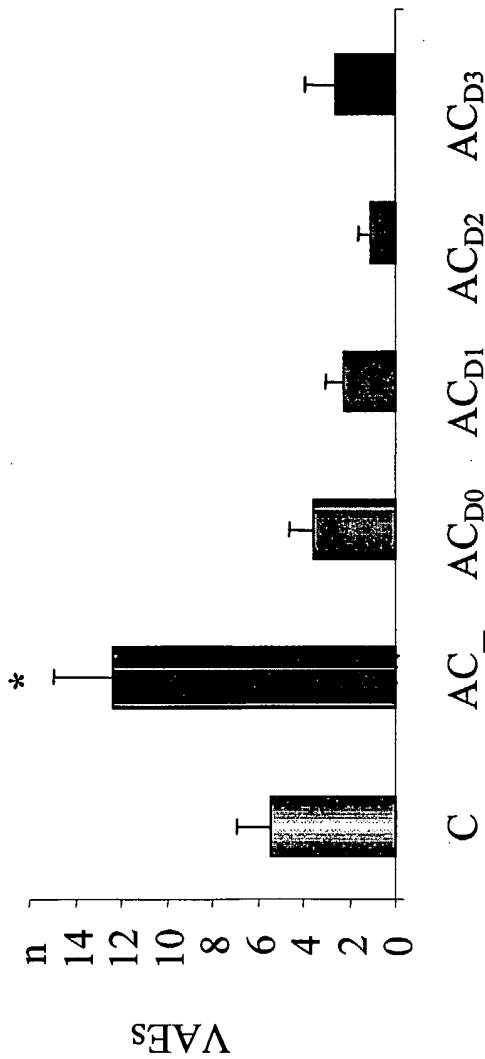


Figure 2: Number of ventricular arrhythmic events (VAEs) expressed as mean ± SEM, during social stress in the different experimental groups.
* significant differences between AC_ and all the other groups.

Membrane capacitance

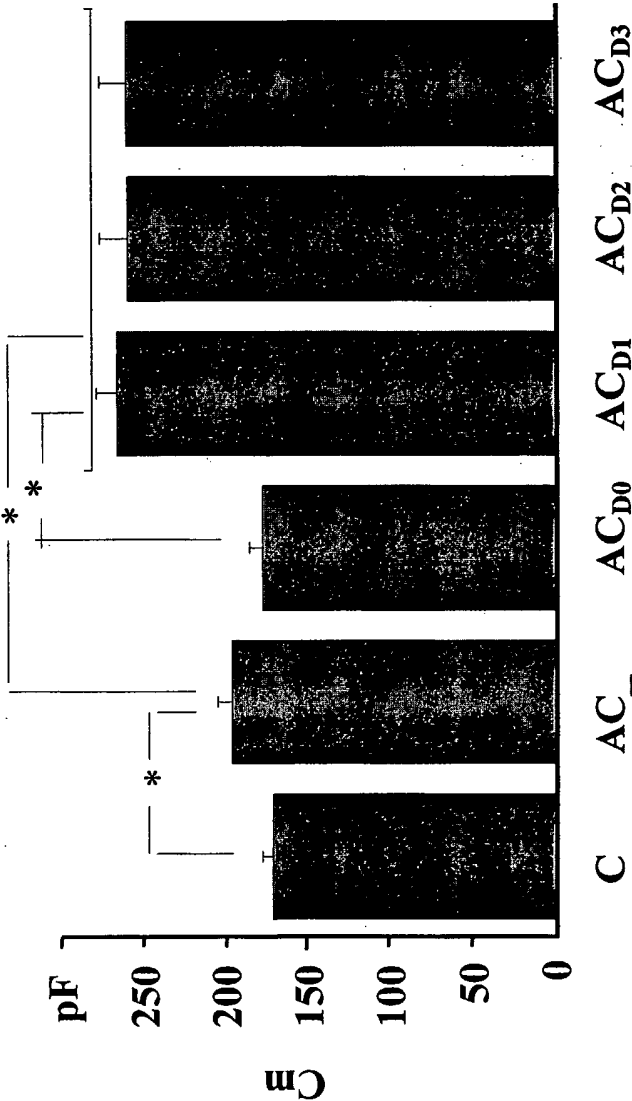


Figure 3: Membrane capacitance (mean $\pm$ SEM) in the 6 experimental groups.
* significant differences among groups

Action Potentials

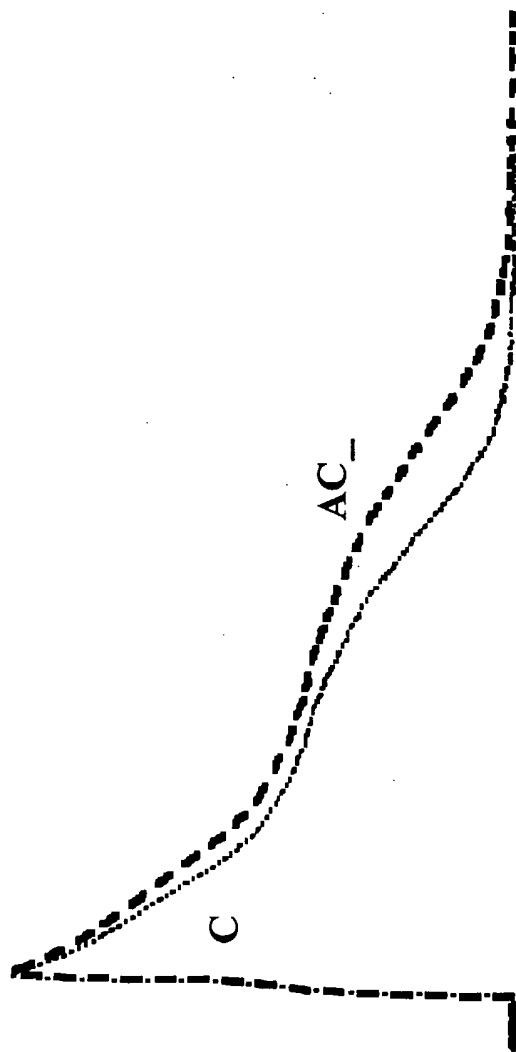
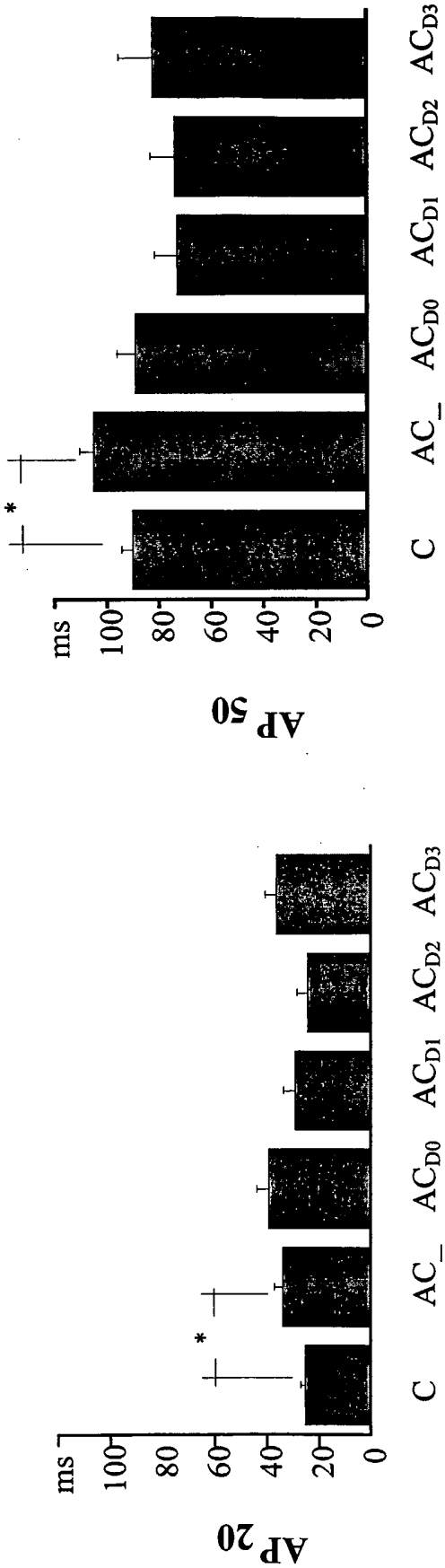


Figure 4: Representative action potentials recorded from cells isolated from normal (C) and banded non-treated (AC_) animals.

Action potential duration



* = p<0.05

Figure 5: APD₂₀ and APD₅₀ in the 6 groups of animals.

Cell diameter and Blood pressure

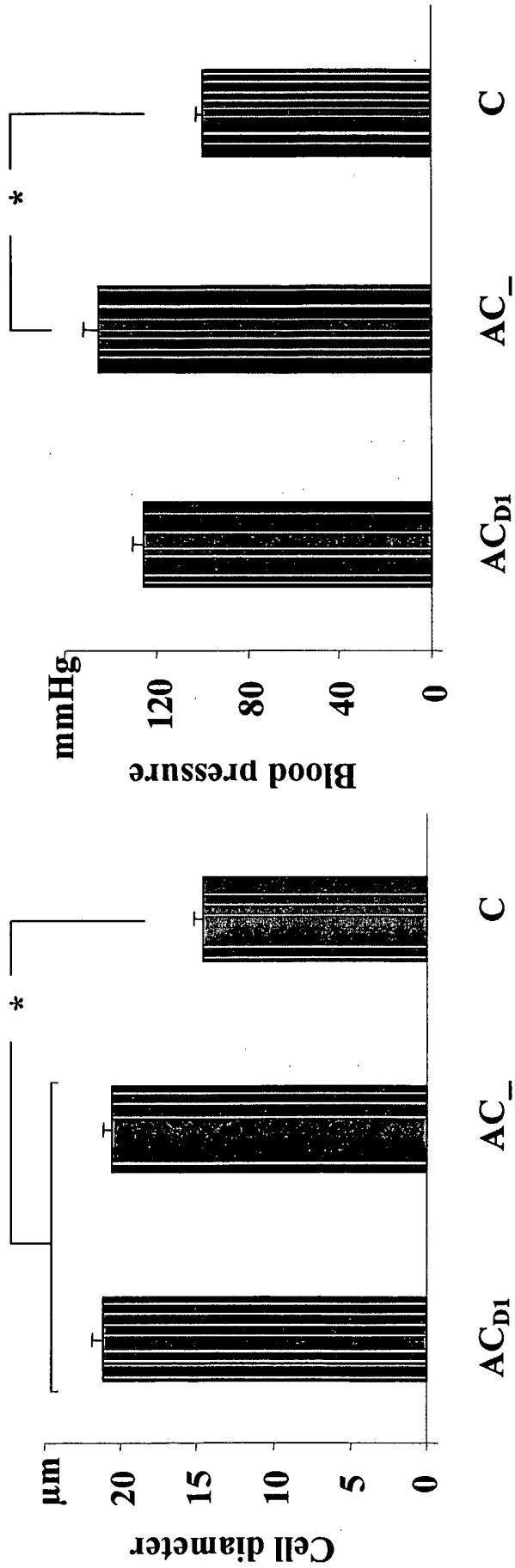


Figure 6: Mean $\pm$ SEM of the transverse cell diameter and blood pressure, in the different experimental groups.
* significant differences between experimental groups

INTERNATIONAL SEARCH REPORT

PCT/EP2005/011879

A. CLASSIFICATION OF SUBJECT MATTER

A61K31/137 A61P9/06

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)

A61K A61P

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 practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ROSSONI ET AL: "The aminotetraline derivative (+)-(R,S)-5,6-dihydroxy-2-methylamino-1,2,3,4-tetrahydro-naphthalene hydrochloride (CHF-1024) displays cardioprotection in postischemic ventricular dysfunction of the rat heart." JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, vol. 307, no. 2, November 2003 (2003-11), pages 633-639, XP002364959 page 307, left-hand column, paragraph 2	1,2
Y	US 2002/045565 A1 (ROBICHAUD ANNETTE) 18 April 2002 (2002-04-18) paragraph '0003!	1,2

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Further documents are listed in the continuation of Box C.

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See patent family annex.

* Special categories of cited documents :

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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

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Date of the actual completion of the international search

30 January 2006

Date of mailing of the international search report

22/02/2006

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INTERNATIONAL SEARCH REPORT

PCT/EP2005/011879

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2002045565	A1	18-04-2002	NONE