POTASSIUM CANTRENOATE, AN ALDOSTERONE RECEPTOR ANTAGONIST, REDUCES ISOPRENALINE INDUCED CARDIAC FIBROSIS IN THE RAT.

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Running title: Potassium canrenoate reduces cardiac fibrosis in the rat

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Abstract

The purpose of the present study was to determine whether or not the administration of an antagonist of aldosterone could prevent the fibrosis induced by an acute injection of isoprenaline. Male rats Wistar were submitted to one subcutaneous injection of isoprenaline (400 mg/kg) and were simultaneously treated with potassium canrenoate in drinking water (20 mg/kg/day) start five days before the injection of isoprenaline. Two months later, echocardiographic and hemodynamic measurements were performed. Then, the heart were prepared for morphometric histology and quantification of fibrosis in the left ventricle. Heart and left ventricular weights were increased significantly by isoprenaline. Potassium canrenoate attenuated this increase. The administration of isoprenaline increased significantly end diastolic diameter and end systolic volume compared to control. These changes were increased further with the addition of potassium canrenoate. In contrast, the fibrosis induced by isoprenaline was reduced significantly by potassium canrenoate at the three section levels. Potassium canrenoate attenuated the fibrosis but not the enhanced dilatation of the left ventricle induced by isoprenaline.
The treatment of congestive heart failure has greatly improved with the introduction of inhibitors of angiotensin I converting enzyme (ACE), beta-adrenergic blockers and blockade of aldosterone receptors by spironolactone, to judge not only from studies on experimental models of the disease but also from large scale randomised clinical trials (Mason et al., 1979; DiBianco, 1990; RALES 1996; Sleight, 2002). These studies suggest that the neuro-hormonal compensatory mechanisms which take place during the development of heart failure, in particular the activation of the renin-angiotensin-aldosterone system and the chronic increase in sympathetic tone (CIBIS-II, 1999) have long term deleterious effects.

Activation of the sympathetic system results in greater release of norepinephrine, which in turn stimulates the beta-adrenoceptors of the heart, especially the beta 1 adrenoceptors (Remme, 1986; van Zwieten and de Jonge, 1986). Such stimulation increases myocardial contractility and tends to restore “normal” hemodynamic conditions despite the deep alteration in cardiac function. This initial benefit is however counterbalanced by the induced increase in cardiac work and the potential chronic ischemia, which progressively leads to loss of contractile tissue, apparition of reparative fibrosis and further alteration of left ventricular function.

The stimulation of beta-adrenergic receptors on fibroblasts and the resulting increase in cAMP level can directly induce myocardial fibrosis (Kahn et al., 1969) in particular of the interstitial type. This fibrosis may also involve the action of the angiotensin II and aldosterone receptors. Indeed, both aldosterone and angiotensin II can induce cardiac fibrosis (Brilla and Weber, 1992; Brilla et al., 1994). Since beta-adrenergic stimulation induces renin release and therefore increases the plasma levels of angiotensin II and aldosterone, part of the fibrotic process induced by beta-adrenergic stimulation could be related to the overproduction of aldosterone and angiotensin II. Therefore, the aim of the present study was to determine,
whether or not aldosterone receptor blockade with potassium canrenoate reduces the cardiac remodeling and fibrosis induced by the acute administration of isoprenaline in the rat.
MATERIALS AND METHODS

Animals
Normotensive male Wistar rats (body weight 300-324 g, age: 8 weeks) were purchased from CERJ (Saint Berthevin, France). All the procedures and protocols involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (Council directive # 87.848, October 19, 1987, Ministère de l’Agriculture et de la Forêt, Service vétérinaire de la Protection Animale, permission # 0299 to MH), and were approved by the institutional animal care committee.

Characterization of the experimental model

Dose of isoprenaline
The experimental model used was a modification of that proposed by Grimm et al (Grimm et al., 1998), inducing cardiomyopathy with subcutaneous injection of high doses of isoprenaline in the female rat. In a preliminary study, the most effective dose inducing a significant myocardial fibrosis two months after the injection of isoprenaline was determined: the animals (male Wistar rats) received one, two or three subcutaneous injections of isoprenaline with an interval of one week between the injections. Three doses of isoprenaline were tested: 150, 200 and 400 mg/kg. Extent of cardiac fibrosis (preferential location at the apex and at the endocardial level) was very variable for each dose level but clearly increased with isoprenaline dose. Therefore in order to induce the largest extent of cardiac fibrosis in the surviving animals, the 400 mg/kg was chosen. Thus, this dose was selected for the present study despite a high mortality rate which was not considered as a limitation for this study.

Telemetry in conscious rats
The amplitude and duration of the effects of the selected subcutaneously administrated 400 mg/kg isoprenaline dose was evaluated by recording heart rate in three additional surviving
conscious rats using telemetry captors (Physio Tel® Telemetry System ETA-F20, DATA Sciences International, USA) placed into the abdominal cavity and fixed by ligature. The administration of isoprenaline was performed fifteen days after surgery. Recordings of the electrocardiogram were obtained for three minutes repeated each day three times (at 10 h, 13 h and 16 h) with a specific acquisition board connected to Snap Master software. Recordings were interpreted with DADISP software (DATA science telemetric system).

**Beta-adrenergic responsiveness**

In order to study the level of desensitisation of cardiac beta-adrenoceptors two months after the acute administration of high dose isoprenaline, dose-response curves to isoprenaline on heart rate (0.0025 to 10 µg/kg) were obtained in control rats (n=7) and in rats having received 400 mg/kg of isoprenaline (n=12). The animals were anaesthetized with pentobarbital and the left femoral vein was cannulated for isoprenaline injections. Heart rate response was studied using ECG recording (Gould RS 3200). The EC\textsubscript{50} and the maximal effect of isoprenaline were determined using an Emax model (Sigma Plot software).

**Choice of potassium canrenoate dose (PC)**

In order to study the effects of blockade of cardiac aldosterone receptors on the isoprenaline-induced fibrosis, potassium canrenoate was used as aldosterone antagonist (20mg/kg/day in drinking water) (Araki et al., 1995). Such dose completely inhibits cardio-vascular effects of aldosterone. However, since extra-cardiac effects of potassium canrenoate, in particular the diuretic effects, could participate indirectly to the cardiac remodeling, we wanted to exclude such diuretic effects and checked that at the chosen dose of potassium canrenoate, no diuretic effect was induced in normal rats. Thus, twelve rats were submitted to a one week treatment with 20 mg/kg of potassium canrenoate in drinking water. At the end of such treatment period, animals were placed in metabolic cages during three hours for diuresis, natriuresis and kaliuresis measurements compared to a control group (n=12). No significant modification of
diuresis, natriuresis and kaliuresis was observed with this selected dose of potassium canrenoate in those normal rats (data not shown). Thus, with 20 mg/kg of potassium canrenoate, we can assume that the observed cardiovascular do not depend on a diuretic action.

**Study design and evaluation parameters**

*Study design*

Animals were divided into four groups: two control groups with or without potassium canrenoate, and two groups of rats submitted to isoprenaline (one subcutaneous dose of 400 mg/kg = ISO) with or without potassium canrenoate which was initiated five days before injection of isoprenaline. The rats were followed for eight weeks and then non invasive and invasive cardiovascular investigations were carried out before sacrifice of animals. The number of animals in each group was 12, 10, 25 and 25 respectively for control, control+PC, iso 400 and iso 400+PC group.

*Non invasive measurements*

The left ventricular dimensions were assessed in vivo under pentobarbital anaesthesia (50 mg/kg intraperitoneally) by echocardiographic examination using a linear probe emitting ultrasounds at 7-10 MHz (ACUSON 128 XP, Acuson Corporation, Mountain View, California, USA). The two-dimensionally guided M-mode recording of the left ventricle provided the measurements of left ventricle cavity dimensions and wall thicknesses: internal end-diastolic diameters (EDD), end-systolic diameters (ESD) and fractional shortening (FS=((EDD-ESD)*100)/EDD), diastolic and systolic thicknesses of septum and posterior wall. All measurements were the mean of at least three independent measures.
Invasive measurements

Once echocardiographic measurements were performed, the animals still being under pentobarbital anaesthesia, a micro-tip pressure transducer catheter (Millar Instruments, 2-french) was inserted into the right carotid artery and connected to a Gould recorder (Model SR 3200, Gould Instruments Co.). The following pressures were obtained: aortic blood pressure, left ventricular pressure and maximal positive and minimal negative left ventricle dP/dt. The heart rate was determined from the ventricular pressure tracing.

Morphometric analysis

After completion of hemodynamic measurements, the heart was removed rapidly, weighed and the heart to body weight ratio was calculated. All ventricles were immersed into 10% buffered formalin, dehydrated in 95° ethanol and then in acetone. They were impregnated with methyl salicylate and embedded in paraffin. Three sections of 6 µm were obtained: one at the basis, one at the middle of the ventricles and one at the apex.

Quantification of fibrosis (collagen extent)

Sirius red staining was used to characterize collagen tissue (Junqueira et al., 1978). Sirius red stained slides of left ventricle were placed under a 3CCD colour camera (KY-F55B, JVC, Japan) which was connected to a quantimeter Qwin (Leica, Rueil Malmaison, France) with the acquisition software Leica Win (version 2.2, Leica Microsystems, France). The extent of fibrosis was determined by quantification of the red intensity and was expressed as a percentage of the entire area of the left ventricle.

Hormonal dosages

At the time of sacrifice, blood samples were taken, plasma was extracted after centrifugation (4°C during 10 minutes, 4000g) for determination of plasma levels of renin activity, angiotensin I and aldosterone by radio-immuno assay using commercially available kits (Schaison et al., 1996).
Drugs

Isoprenaline (dl Isoproterenol hydrochloride) and potassium canrenoate were purchased from Sigma (Saint Quentin Fallavier, France). Sodium pentobarbital (Sanofi Santé Animal, France) was used in injectable solution. Isoprenaline was prepared under sterile conditions with distilled water.

Statistical analysis

The statistical analysis of each parameter was carried out with a two-factor variance analysis using as factors: isoprenaline administration and potassium canrenoate administration. For the analysis of extent of fibrosis, a third factor was used: the level of the section (basis, medium, and apex). Between group comparisons were performed using a Newman-Keuls test. For comparison of mortality rates between the different groups, a Chi-square test was used. P values of less than 0.05 were considered to be statistically significant.
RESULTS

Preliminary experiments: cardiac effects of isoprenaline

Telemetry, Holter recording

The subcutaneous injection of isoprenaline (400 mg/kg) induced an important increase in heart rate (Fig. 1), from 358 ± 2 beats per minute at baseline to 536 ± 14 beats per minutes five minutes after the first injection of isoprenaline. Heart rate returned to baseline values three days after injection (358 ± 14 beats per minute).

Dose-response curve to isoprenaline (Fig. 2)

The maximal chronotropic effect of isoprenaline two months after the acute isoprenaline administration was reduced: for the control group, Emax (heart rate maximal increase) was 133 ± 27 beats per minute and the EC50 was 0.02 ± 0.02 µg/kg and for the isoprenaline treated group, Emax was 84 ± 18 beats per minute, EC50 was 0.01 ± 0.01 µg/kg. Difference between groups were significant for Emax (p<0.001) but not for EC50.

Mortality rate

During the two months of study duration, no death was recorded in the two control groups but 7 animals died in the isoprenaline treated group (27% death rate) and 15 died in the isoprenaline+potassium canrenoate treated group (52% death rate), p=0.07 between these last two groups (Chi square test).

Some animals died immediately after onset of anaesthesia or during hemodynamic investigation explaining the different group numbers for analysis of non invasive and invasive cardiovascular measurements.
Morphological parameters (Table 1)

Heart and left ventricular weights were increased significantly by isoprenaline. Such increase was prevented partly by the administration of potassium canrenoate.

Echocardiographically measured at the level of end of mitral valve chordae (mid-ventricle) end diastolic diameter and end-systolic diameter were increased significantly in the isoprenaline treated group and such enlargement was enhanced further by administration of potassium canrenoate. Between these last two groups (Isoprenaline and Isoprenaline + potassium canrenoate), the difference was however significant only for EDD (p<0.003). The left ventricular shortening fraction was significantly reduced by the administration of isoprenaline compared to control groups.

Hemodynamic parameters (Table 2)

No significant difference was found between groups for the systolic, diastolic and mean arterial blood pressures, the left ventricular systolic and end-diastolic pressures, heart rate or the dP/dt+. A significant reduction of dP/dt - was observed in the Isoprenaline + potassium canrenoate group compared with the control group.

Extent of fibrosis

The left ventricular area was significantly increased with potassium canrenoate and isoprenaline alone or in combination (Fig. 3). The extent of fibrosis was significantly increased in the isoprenaline treated group but such increase was partly but significantly prevented by potassium canrenoate at the three levels studied (Fig. 4). Isoprenaline-induced fibrosis preferentially developed at the apex compared with the basal level (p<0.001).
Plasma neuro-hormone concentrations (Table 3)

Plasma renin activity and plasma angiotensin I levels were increased significantly in rats submitted initially to the injection of isoprenaline. No statistical difference was recorded between the other groups.

Plasma levels of aldosterone were elevated significantly in the group of animals submitted to isoprenaline administration and treated with potassium canrenoate (p<0.05 versus isoprenaline treated group).
DISCUSSION

The present study shows that antagonism of aldosterone receptors with potassium canrenoate interacts with cardiac remodeling following the acute administration of high doses of isoprenaline. The drug reduces the extent of fibrosis and such effect is associated with a greater left ventricular enlargement.

Isoprenaline is a non selective agonist of beta 1 and beta 2 adrenoceptors, and can exert toxic effects by several mechanisms including intracellular cAMP increase, calcium overload (Dhalla et al., 1996), alteration of electrophysiological properties of cardiomyocytes (Hart, 1994), ischemia and increased oxidative stress (Bindoli et al., 1992; Remiao et al., 2001). The two latter can lead to the destruction of myocytes either by apoptosis or necrosis, with loss of contractile tissue, fibrosis (reparative fibrosis) and severe arrhythmias. Indeed, following the acute administration of such high doses of isoprenaline, a trend toward a mortality increase was observed, whether isoprenaline was injected alone or in association with potassium canrenoate. The acute administration of isoprenaline induces a cardiac remodeling process with a moderate degree of cardiac hypertrophy and dilatation, as previously described (Grimm et al., 1998), with an important interstitial fibrosis especially at the subendocardial and apical cardiac levels, in confirmation of earlier studies (VanVleet et al., 1983). Ng et al have shown that the subendocardial layers of the left ventricle are more sensitive to low doses of isoprenaline than the epicardial layer. At the same dose (5 mg/kg) of isoprenaline, the necrosis was tenfold higher at the endocardial level than that at epicardial level (Ng et al., 2002). The density of adrenoceptors is seven-to eightfold higher in the heart than in skeletal muscle (Rothwell et al., 1987). A high density of the beta adrenoceptors has been observed in the endocardial layers of the heart (Murphree and Saffitz, 1989). The abundance of these receptors can explain the larger response of the heart to isoprenaline. One major mechanism underlying this cardiac toxicity is likely to be the myocardial ischemia secondary to the severe
increase in myocardial oxygen consumption during beta-adrenergic stimulation, which is
maintained during 48 hours according to the Holter recordings obtained in the present study.
Indeed, both the chronotropic and the inotropic actions of beta-adrenergic stimulation impact
on cardiac oxygen consumption and have the potential to induce sustained ischemia, leading
to necrosis of myocytes and reparative fibrosis (Benjamin et al., 1989). However, reparative
fibrosis may not be the only mechanism for the observed fibrosis since a direct activation of
beta-adrenergic receptors of fibroblasts by isoprenaline may induce collagen synthesis. Thus,
two types of fibrosis can occur: reactive fibrosis involving the intramyocardial, perivascular
or interstitial spaces, and reparative fibrosis, which is a scar tissue replacing necrotic
cardiomyocytes (Weber et al., 1990). Since fibroblasts can be also activated by angiotensin II
and aldosterone, and since beta adrenergic stimulation increases renin release by the juxta-
glomerular apparatus (van Zwieten and de Jonge, 1986; Vatta et al., 1992), the objective of
the present study was to test the interaction between beta-adrenergic stimulation and
aldosterone action.

The present experiments show that blockade of aldosterone receptors with potassium
canrenoate partly reduces the isoprenaline-induced fibrosis. The present results confirm
previous experiments by Gallego et al who also found that spironolactone significantly reduce
isoprenaline-induced fibrosis but not captopril (Gallego et al, 2001). Their experimental
conditions were however different and spironolactone was only active at very high doses (200
mg/kg subcutaneously). The effectiveness of potassium canrenoate at 20 mg/kg in drinking
water in our experiments is in favour of a better bioavailability compared to that of
spironolactone (used in Gallego experiments) and which is extremely difficult to solubilize at
these concentrations. Grimm et al reported only a transient increase in components of the
renin-angiotensin system after isoprenaline and a partial inhibition of cardiac fibrosis by
ramipril (Grimm et al, 1998). The results of Gallego et al, Grimm et al and ours argue in
favour of a major role for mineralocorticoid receptor stimulation in the isoprenaline-induced cardiac fibrosis secondary to fibroblast stimulation in addition to the reparative fibrosis. They also suggest that part of the isoprenaline-induced fibrosis is mediated by circulating aldosterone resulting from beta-adrenergic induced renin release. Aldosterone may potentiate catecholamines by reducing their uptake from the extracellular space, which is an essential step for the disposition of adrenaline and noradrenaline (Yamamoto et al., 1976). Aldosterone stimulates fibroblasts and collagen synthesis by activating metalloproteinases (Funck et al., 1997). In particular, aldosterone induces an increase of messenger RNA for type I and III collagen in myocytes in both ventricles (Robert et al., 1994).

To judge from experiments in rats and in humans, tissues other than the adrenal gland can produce aldosterone including the heart itself (Silvestre et al., 1998), vascular smooth muscle (Takeda et al., 1995) and the brain (Gomez-Sanchez et al., 1997). However, the relative importance of the extra-adrenal gland production of aldosterone remains unknown and the adrenal gland remains the major source of aldosterone since adrenalectomy almost abolishes the plasma levels of the hormone (Rocha et al., 2000).

Potassium canrenoate is a competitive antagonist of aldosterone, used as a diuretic in the treatment of hypertension. Although spironolactone appears to have beneficial therapeutic effects in humans as demonstrated in the RALES study by inhibiting the effects of aldosterone on cardiac remodeling, potassium canrenoate, its main metabolite may have direct toxic effects. Indeed, potassium canrenoate is genotoxic (Martelli et al., 2002) and induces DNA damage in different tissues including the liver, thyroid, brain and mammary gland (Cook et al., 1988). Studies in primary cultures of rat hepatocytes demonstrate that potassium canrenoate induces DNA fragmentation and DNA repair (Martelli et al., 1999). These potential toxic effects of potassium canrenoate may explain the observed trend of survival reduction in the rats treated with combination of potassium canrenoate and isoprenaline.
Together with the partial inhibition of fibrosis, the treatment with potassium canrenoate was associated with a greater enlargement of the left ventricle, as shown by echocardiographic measurements of left ventricular dimensions in vivo. These findings may have important implications in the treatment of heart failure since an accurate balance must be obtained between the prevention of fibrosis and prevention of left ventricular dilatation. Therefore, the combined actions of angiotensin II and aldosterone may represent a relevant mechanism for remodeling of the extracellular matrix in the myocardium.

In conclusion, the present study shows that the acute administration of high doses of isoprenaline induces a cardiac remodeling with dilatation and fibrosis of the left ventricle which is partly reduced by an antagonist of aldosterone receptors. The potential role of the interaction between beta-adrenergic stimulation and aldosterone remains to be established in human heart failure where both the sympathetic tone and the renin-angiotensin-aldosterone system are activated as compensatory mechanisms. The present results argue in favour of the synergic therapeutic action of blockade of both beta-adrenergic and aldosterone receptors in heart failure. No randomised trial has yet however tested in chronic heart failure the benefit risk ratio of a systematic combination of beta-blockers and aldosterone antagonist.
References


Spironolactone and captopril attenuates isoproterenol-induced cardiac remodelling in rats.

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Footnotes

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**Legends for figures**

**Fig. 1.** Telemetry, Holter recording. Three rats received a dose of isoprenaline (400 mg/kg) by subcutaneous injection. The recording were performed one day before injection of isoprenaline and continued during one week. Means±S.E.M.

**Fig. 2.** Dose-response curve to isoprenaline. Two groups of rats were studied during two months. A control group (♦, n=7) and a group receiving isoprenaline at a dose of 400 mg/kg (■, n=11). Variation of heart rate were determined. Means±S.E.M.

**Fig. 3.** Measurements of the area of the left ventricle in the four groups of animals after administration of isoprenaline (400 mg/kg) and potassium canrenoate (20 mg/kg/day). Control, n=12; Control+pc, n=10, Iso 400, n=18 and Iso+pc, n=12

Bars are presented as means ± S.E.M.. Analysis of variance (ANOVA) was performed to determine the statistical significance of differences caused by administration of isoprenaline, potassium canrenoate and their association. ISO: isoprenaline, PC: potassium canrenoate

F test: p<10⁻⁶ for isoprenaline

F test: p<10⁻⁶ for potassium canrenoate

F test: p<10⁻⁶ for interaction of these two factors

a: Control group versus Control+pc group

b: Control group versus Iso 400 group

c: Control group versus Iso400+pc group

d: Control+pc group versus Iso 400+pc group

*: Iso 400 group versus Iso 400+pc group
**Fig. 4.** Measurements of the area of the fibrosis of the left ventricle in the four groups of animals after administration of isoprenaline (400 mg/kg) and potassium canrenoate (20 mg/kg/day).

Bars are presented as means ± S.E.M.. Analysis of variance (ANOVA) was performed to determine the statistical significance of differences caused by administration of isoprenaline, potassium canrenoate and their association. ISO: isoprenaline, PC: potassium canrenoate

Control, n=12; Control+pc, n=10, Iso 400, n=18 and Iso+pc, n=12

F test: p<10^{-6} for isoprenaline

F test: p=0.04 for potassium canrenoate

F test: p=0.03 for interaction of these two factors

b: Control group versus Iso 400 group
c: Control group versus Iso400+pc group
d: Control+pc group versus Iso 400+pc group
e: Iso 400 group versus Iso 400+pc group
f: Iso 400 group versus Control+pc group
Table 1

Selected morphological parameters in rats submitted to isoprenaline (400 mg/kg) and potassium canrenoate (20mg/kg/day)

<table>
<thead>
<tr>
<th>Groups</th>
<th>BW (g) ± S.E.M.</th>
<th>HW (g) ± S.E.M.</th>
<th>LV (g) ± S.E.M.</th>
<th>HW/BW (g/kg) ± S.E.M.</th>
<th>LVESD (mm) ± S.E.M.</th>
<th>LVEDD (mm) ± S.E.M.</th>
<th>SF (%) ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>503±17</td>
<td>1.30±0.04</td>
<td>1.16±0.04</td>
<td>2.59±0.05</td>
<td>5.4±0.1</td>
<td>8.6±0.1</td>
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<td>n=12</td>
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<tr>
<td>Control+PC</td>
<td>555±8</td>
<td>1.32±0.03</td>
<td>1.20±0.03</td>
<td>2.39±0.06</td>
<td>4.7±0.2</td>
<td>8.2±0.2</td>
<td>42.8±1.5</td>
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<td>n=10</td>
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<tr>
<td>Iso 400</td>
<td>531±15</td>
<td>1.56±0.04</td>
<td>1.40±0.03</td>
<td>3.03±0.13</td>
<td>6.3±0.3</td>
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<td>n=18</td>
<td>p&lt;0.0004</td>
<td>p&lt;0.0003</td>
<td>p&lt;0.02</td>
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<td>p&lt;0.0005</td>
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<td>p&lt;0.005</td>
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<td>p&lt;0.003</td>
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<tr>
<td>Iso 400+PC</td>
<td>510±12</td>
<td>1.46±0.07</td>
<td>1.31±0.05</td>
<td>2.85±0.11</td>
<td>7.1±0.4</td>
<td>10.5±0.3</td>
<td>32.3±1.7</td>
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<td>n=12</td>
<td>p&lt;0.05</td>
<td>p&lt;0.0002</td>
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</table>

Results are expressed as mean ± S.E.M.

BW: body weight, HW: heart weight, LVW: left ventricular weight, HW/BW: heart weight to body weight ratio, LVESD: left ventricular end-systolic diameter; LVEDD: left ventricular end-diastolic diameter, SF: shortening fraction, ISO: isoprenaline, PC: potassium canrenoate

b: Control group versus Iso 400 group
c: Control group versus Iso400+pc group
d: Control+pc group versus Iso 400+pc group
e: Iso 400 group versus Iso 400+pc group
f: Iso 400 group versus Control+pc group
Table 2

Hemodynamic parameters in rats submitted to isoprenaline (400 mg/kg) and potassium canrenoate (20 mg/kg/day)

<table>
<thead>
<tr>
<th>Group</th>
<th>HR  (min⁻¹)</th>
<th>SAP (mmHg)</th>
<th>DAP (mmHg)</th>
<th>MAP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>dP/dt max (mmHg/s)</th>
<th>dP/dt min (mmHg/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>405±12</td>
<td>148±5</td>
<td>120±3</td>
<td>134±4</td>
<td>0.6±1.3</td>
<td>8542±459</td>
<td>8452±357</td>
</tr>
<tr>
<td>Control+PC</td>
<td>365±17</td>
<td>147±8</td>
<td>121±6</td>
<td>134±6</td>
<td>3.8±2.7</td>
<td>7542±780</td>
<td>6538±491</td>
</tr>
<tr>
<td>ISO 400</td>
<td>412±16</td>
<td>146±7</td>
<td>120±6</td>
<td>134±6</td>
<td>2.2±2.4</td>
<td>6954±558</td>
<td>6463±500</td>
</tr>
<tr>
<td>ISO 400+PC</td>
<td>367±12</td>
<td>144±11</td>
<td>118±10</td>
<td>132±11</td>
<td>1.2±1.8</td>
<td>7264±428</td>
<td>5620±283</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M.

HR: heart rate, SAP: systolic aortic pressure, DAP: diastolic aortic pressure, MAP: mean aortic pressure, LVEDP: left ventricular end-diastolic pressure, dP/dt max: positive derivated pressure, dP/dt min: negative derivated pressure, ISO: isoprenaline, PC: potassium canrenoate

\( ^d \): Control+pc group versus Iso 400+pc group
Table 3

Plasma levels in rats receiving isoprenaline (400 mg/kg) and potassium canrenoate (20 mg/kg/day)

<table>
<thead>
<tr>
<th>Group</th>
<th>PRA ng/ml/h</th>
<th>AI ng/ml</th>
<th>Aldo pg/ml</th>
<th>NA ng/ml</th>
<th>Ad ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.7±1.2</td>
<td>4.1±0.6</td>
<td>185.8±74.8</td>
<td>353.0±86.3</td>
<td>365.0±124.8</td>
</tr>
<tr>
<td>n=8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control+PC</td>
<td>15.00±1.1</td>
<td>3.6±0.3</td>
<td>133.7±13.8</td>
<td>448.6±90.2</td>
<td>264.3±91.8</td>
</tr>
<tr>
<td>n=10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISO 400</td>
<td>17.0±0.7</td>
<td>5.00±0.3</td>
<td>267.0±18.1</td>
<td>203.8±37.8</td>
<td>90.0±16.0</td>
</tr>
<tr>
<td>n=14</td>
<td>p&lt;0.03b</td>
<td>p&lt;0.01e</td>
<td>p&lt;0.05e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISO 400+PC</td>
<td>17.6±1.6</td>
<td>4.9±0.9</td>
<td>406.3±70.2</td>
<td>262.5±38.0</td>
<td>138.8±38.9</td>
</tr>
<tr>
<td>n=11</td>
<td>p&lt;0.03e</td>
<td>p&lt;0.04d</td>
<td>p&lt;0.02c</td>
<td>p&lt;0.002d</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M.


b: Control group versus Iso 400 group
c: Control group versus Iso400+pc group
d: Control+pc group versus Iso 400+pc group
e: Iso 400 group versus Iso 400+pc group
Figure 1

Duration of recording (days)

Beats/min

D - 1  D 0  D 1  D 2  D 3  D 4

ISOPRENALINE

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Figure 2

Variation of heart rate (beats/min) vs. Concentration of isoprenaline (µg/kg)
Figure 3

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