Data Article

Data set on effect of amaranth proteins on the RAS system. In vitro, in vivo and ex vivo assays

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ABSTRACT

Data set presented in this article is related to the research paper entitled "Effect of amaranth proteins on the RAS system. In vitro, in vivo and ex vivo assays", available in Food Chemistry [1]. In this article, we evaluated the effect on systolic blood pressure of spontaneously hypertensive rats (SHR) of different samples with amaranth proteins/peptides. The effect of these samples on RAS system was evaluated using in vitro and ex vivo assays. The concentration of renin and angiotensin converting enzyme (ACE) was evaluated using two commercial ELISA kits. Renin concentration was estimated through a direct immunoassay and ACE concentration with an immunoassay based on a competitive inhibition. In addition, the ACE inhibitory activity in plasma was evaluated using a spectrophotometric assay according to [2]. Ex vivo experiments were done with thoracic aorta extracted during the surgical procedure employed to obtain blood samples according to [3]. Data presented in this article recollect a very extensive work on how can be affect the RAS system in SHR model using amaranth protein/peptides as potential antihypertensive samples. These data could

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be useful to design novel functional foods for hypertensive individuals.

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Specifications Table

| Subject              | Food Science                                          |
|----------------------|-------------------------------------------------------|
| Specific subject area| Bioactive peptides                                    |
|                      | Antihypertensive peptides                             |
| Type of data         | Table                                                 |
| How data were acquired| A tail cuff and a pulse sensor (NarcoBiosystems, Houston, TX) were used for recording the systolic blood pressure. ELISA test for ACE and renin plasma concentration. Spectrophotometric assay for ACE activity. Contractile response of aortic smooth muscle was measured in a force transducer (Grass FT.03D, Grass Telefactor, West Warwick, CT, USA). |
| Data format          | Raw                                                   |
| Parameters for data collection| Sixty male SHR rats were used weighed between 210 - 290 g and had approximately 10 weeks of age at the beginning of the study. Rats were housed in stainless steel cages. 4 animals per cage, with sterilized bedding. The facility had air conditioning and a 12 h light – 12 h dark cycle. Food and water were provided ad libitum. Tap water was provided in sterilized bottles with stainless steel nipples. Animals were fed with extruded balanced feed. |
| Description of data collection| Animals were divided into 8 groups of 7 – 8 animals each, except captopril and aliskiren groups in which 4 animals were used. Samples were administered by the orogastric route with 2 ml of each sample dispersed in distilled water. The systolic blood pressure was measured according to [3]. After surgical procedure the rat’s abdominal aorta was then cannulated to collect a blood sample (roughly 6 ml) in heparin-coated tubes. The plasma was separated and used for plasma ACE. Renin concentrations and plasma ACE activity. Also, the thoracic aorta was resected and then cut into two mm long rings. The ex vivo assay was performed according to [3]. |
| Data source location | Institution: CIDCA, UNLP, CONICET City/Town/Region: La Plata, Buenos Aires Country: Argentina |
| Data accessibility   | With the article                                      |
| Related research article| Author’s name: Santiago Suárez. Paula Aphalo. Gustavo Rinaldi. María Cristina Añón. Alejandra Quiroga Title: Effect of amaranth proteins on the RAS system. In vitro, in vivo and ex vivo assays Journal: Food Chemistry DOI: 10.1016/j.foodchem.2019.125601 |

Value of the Data

- This data collects in vitro, in vivo and ex vivo information about amaranth protein and peptides with antihypertensive effect. The interaction of the different approaches is important for understanding the mechanism of action of amaranth peptides.
- The data will help to understand the possible mechanism of action of food peptides on the RAS system.
- Data are useful for researchers and academicians to acquire innovative knowledge about the effect of bioactive peptides on RAS system. In addition, data provide new insights and information to consider in the design of novel functional foods with amaranth what could be useful for entrepreneurs and food industry.
- How affect the bioactive peptides on RAS system is a valuable tool to develop a new functional food. Emulsions with amaranth proteins could be a delivery system of antihypertensive peptides with the possibility to enhance the bio-disponibility and reach the target organ successfully.
1. Data description

Data describes the effect of amaranth protein/peptides on RAS system inquiring into the mechanism of action of these samples using in vitro, in vivo and ex vivo approaches [1].

Treatment groups were:

1. G_W: Negative control group. Animals treated with water, which did not receive amaranth proteins.
2. G_C: Captopril group. Animals treated with captopril, an ACE inhibitor.
3. G_A: Aliskiren group. Animals treated with aliskiren, a renin inhibitor.
4. G_API: API group. Animals treated with amaranth protein isolated (API).
5. G_AH: AH group. Animals treated with amaranth protein hydrolysate (AH).
6. G_VIKP: VIKP group. Animals treated with the synthetic peptide VIKP.
7. G_E: w/o Emulsion group. Animals treated with w/o emulsion.
8. G_E+VIKP: w/o Emulsion + VIKP group. Animals treated with w/o emulsion added with VIKP.

In order to compare mean values, a one way analysis of variance (ANOVA) multiple comparisons was applied. The critical significance level was set at p < 0.05. All samples were compared to G_W (negative control group).

Table 1 shows the reduction in SBP values exerted in each experimental group. Data were expressed as the decrease of SBP in mmHg of animals 3 h after the administration of each sample with respect to the SBP measured at the beginning of the experiment (SBP3h-SBP0h). ΔP values are presented as mean ± SEM. Animals belonging to the G_E and G_E+VIKP groups showed the most significant reduction in the SBP reaching values of 42 ± 2 mmHg and 35 ± 2 mmHg respectively. The administration of API, AH or VIKP in water as vehicle (G_API, G_AH y G_VIKP groups) caused a reduction in SBP values that was significantly lower than those observed in the groups mentioned above (25 ± 14 mmHg, 26 ± 3 mmHg and 21 ± 3 mmHg respectively.)

Table 2 shows ACE plasma concentration of different groups assayed. This ELISA immunoassay is based on a competitive inhibition. Calibration curve was calculated according to the manufacturer's directions and the values are as follows: y = 0.7089 e (-0.001405.X) + 0.107 where "y" means OD at 450nm and "x" is ACE sample concentration in µg/ml. It can be observed that G_W group presented extremely low values of ACE levels (0.17 ± 0.02 µg/ml), whereas ACE concentration in G_C, G_A and G_E+VIKP groups were 13.6–25.8 times higher than control group. API, AH and VIKP (G_API, G_AH y G_VIKP groups respectively) induced an increase in the ACE levels that was 7.6 to 5.3 times higher than control group (1.3 ± 0.2 µg/ml, 0.90 ± 0.3 µg/ml and 1.1 ± 0.3 µg/ml respectively. p < 0.05). The same trend has been observed in studies evaluating synthetic drugs in hypertension treatments [4,5].

Table 3 shows renin plasma concentration in the different samples assayed. Calibration curve obtained was y = 0.005x + 0.008 where "y" means OD at 450 nm and "x" represented renin concentration in pg/ml. Only the G_A, G_API and G_AH groups presented differences in plasma renin levels, as compared to G_W group. No differences were found in the levels of this enzyme between G_C, G_E, G_VIKP and G_E+VIKP groups and the control group G_W.

Table 4 shows % ACE activity/µg ACE in plasma collected after treatments. Data are expressed as relative to 100% ACE activity to water control group (G_W). The lowest activity values (4–7% active ACE/µg ACE) corresponded to groups G_C, G_A, G_E and G_E+VIKP whereas the highest activity values were found in G_VIKP, G_AH and G_API groups, which presented 20–13% active ACE/µg ACE. The administration of different samples decreases the enzymatic activity of ACE, together with an increase in plasma levels (Table 2), probably to counter balance the inhibitory effect exerted by the hypotensive peptides.

Table 5 shows the contractile activity determined in presence of potassium ions. Contractile force was higher in G_W, G_C and G_A groups (roughly 0.45 g/mg), whereas this activity was significantly lower in the animals belonging to groups G_API, G_VIKP, G_E+VIKP, G_AH and G_E (0.34–0.29 g/mg). Upon treating aorta rings with physiological concentrations of norepinephrine, statistically differences were observed in G_VIKP and G_E+VIKP groups.
Table 1
Systolic blood pressure before and after treatment. ΔP (SBP<sub>3h</sub>-SBP<sub>0hi</sub>) values are presented as mean ± SEM.

| Treatment group | SBP (mm Hg) | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 |
|-----------------|-------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| GW              |             | 196| 281| 215| 190| 188| 206|    |    |    |    |    |    |    |    |
|                 |             | 176| 280| 193| 195| 177| 196|    |    |    |    |    |    |    |    |
|                 |             | 200| 273| 244| 202| 170| 201|    |    |    |    |    |    |    |    |
| Average         |             | 191| 278| 217| 196| 178| 201|    |    |    |    |    |    |    |    |
| post intragastric administration | 201| 314| 208| 228| 167| 188|    |    |    |    |    |    |    |    |    |
|                 |             | 186| 283| 179| 222| 152| 187|    |    |    |    |    |    |    |    |
|                 |             | 196| 244| 156| 203| 162| 250|    |    |    |    |    |    |    |    |
| Average         |             | 194| 280| 181| 218| 160| 208|    |    |    |    |    |    |    |    |
| ΔP = SBP<sub>3h</sub>-SBP<sub>0hi</sub> | 4 | 2 | -36 | 22 | -18 | 7 |    |    |    |    |    |    |    |    |    |
| ΔP average      |             | -3<sup>a</sup>|    |    |    |    |    |    |    |    |    |    |    |    |    |
| SEM             |             | 8 |    |    |    |    |    |    |    |    |    |    |    |    |    |
| GA              |             | 196| 185| 195| 196|    |    |    |    |    |    |    |    |    |    |
|                 |             | 197| 183| 187| 192|    |    |    |    |    |    |    |    |    |    |
|                 |             | 190| 186| 185| 193|    |    |    |    |    |    |    |    |    |    |
| Average         |             | 194| 185| 189| 194|    |    |    |    |    |    |    |    |    |    |
| post intragastric administration | 145| 156| 139| 139|    |    |    |    |    |    |    |    |    |    |    |
|                 |             | 147| 154| 140| 135|    |    |    |    |    |    |    |    |    |    |
|                 |             | 152| 150| 145| 140|    |    |    |    |    |    |    |    |    |    |
| Average         |             | 148| 153| 141| 138|    |    |    |    |    |    |    |    |    |    |
| ΔP = SBP<sub>3h</sub>-SBP<sub>0hi</sub> | -46 | -31 | -48 | -56 |    |    |    |    |    |    |    |    |    |    |    |
| ΔP average      |             | -45<sup>b</sup>|    |    |    |    |    |    |    |    |    |    |    |    |    |
| SEM             |             | 5 |    |    |    |    |    |    |    |    |    |    |    |    |    |
| GC              |             | 208| 205|    | 205|    |    |    |    |    |    |    |    |    |    |
|                 |             | 199| 205|    | 193|    |    |    |    |    |    |    |    |    |    |
|                 |             | 199| 205|    | 183|    |    |    |    |    |    |    |    |    |    |
| Average         |             | 202| 205|    | 194|    |    |    |    |    |    |    |    |    |    |
| post intragastric administration | 166| 171|    | 168|    |    |    |    |    |    |    |    |    |    |    |
|                 |             | 159| 173|    | 171|    |    |    |    |    |    |    |    |    |    |
|                 |             | 162| 177|    | 163|    |    |    |    |    |    |    |    |    |    |
| Average         |             | 162| 174|    | 167|    |    |    |    |    |    |    |    |    |    |
| ΔP = SBP<sub>3h</sub>-SBP<sub>0hi</sub> | -40 | -31 | -48 | -66 |    |    |    |    |    |    |    |    |    |    |    |
| ΔP average      |             | -32<sup>b</sup>|    |    |    |    |    |    |    |    |    |    |    |    |    |
| SEM             |             | 4 |    |    |    |    |    |    |    |    |    |    |    |    |    |
| GAPI            |             | 166| 162| 197| 180| 259|    |    |    |    |    |    |    |    |    |
|                 |             | 160| 166| 208| 187| 238|    |    |    |    |    |    |    |    |    |
|                 |             | 159| 168| 204| 190| 234|    |    |    |    |    |    |    |    |    |
| Average         |             | 162| 165| 203| 186| 244|    |    |    |    |    |    |    |    |    |
| post intragastric administration | 153| 155| 136| 140| 179|    |    |    |    |    |    |    |    |    |    |
|                 |             | 145| 157| 147| 154| 173|    |    |    |    |    |    |    |    |    |
|                 |             | 158| 154| 152| 162| 182|    |    |    |    |    |    |    |    |    |
| Average         |             | 152| 155| 145| 152| 178|    |    |    |    |    |    |    |    |    |
| ΔP = SBP<sub>3h</sub>-SBP<sub>0hi</sub> | -10 | -10 | -58 | -34 | -66 |    |    |    |    |    |    |    |    |    |    |
| ΔP average      |             | -35<sup>b</sup>|    |    |    |    |    |    |    |    |    |    |    |    |    |
| SEM             |             | 12 |    |    |    |    |    |    |    |    |    |    |    |    |    |
| GAH             |             | 181| 184| 184| 162| 159| 174| 203| 199| 208| 183| 162| 168| 170| 195|
|                 |             | 184| 184| 186| 159| 161| 179| 192| 184| 198| 173| 166| 182| 181| 191|
|                 |             | 158| 154| 152| 162| 182|    |    |    |    |    |    |    |    |    |
| Average         |             | 182| 182| 186| 160| 161| 177| 195| 185| 202| 175| 163| 176| 178| 190|
| post intragastric administration | 145| 166| 165| 145| 140| 156| 167| 176| 146| 123| 148| 150| 146|    |    |
|                 |             | 156| 159| 166| 156| 145| 158| 161| 177| 148| 114| 141| 158| 148|    |    |
|                 |             | 156| 168| 158| 151| 146| 155| 168| 177| 146| 129| 145| 157| 139|    |    |
| Average         |             | 152| 164| 163| 151| 144| 156| 165| 177| 147| 123| 145| 155| 144|    |    |
2. Experimental design. Materials and methods

2.1. Samples

The following samples were used for in vivo assays:

- Amaranth protein isolate (API) and hydrolysate (AH) prepared from *Amaranthus hypochondriacus* as described elsewhere [2]. The protein content was 87 ± 1 and 57 ± 2% w/w w.b. for API and AH respectively.

- VIKP peptide, which is a synthetic peptide from 11S amaranth protein. This peptide has inhibitory activity on ACE [6].

- O/W 20:80 emulsions prepared with sunflower oil and 1:1 protein mixture of API and AH at pH 2 with a total protein concentration of 2% w/v with or without VIKP peptide [(API50 + AH50)-2%+ VIKP and (API50 + AH50)-2%, respectively].
Emulsions were prepared according to [2]. Emulsions were frozen at \(-80\, ^\circ\text{C}\), lyophilized and resuspended as required. Before administration, the resuspended emulsions were homogenized with a magnetic stirring bar.

- Commercial ACE and renin inhibitors (captopril and aliskiren, respectively) were employed as positive controls.

2.2. In vivo assays

2.2.1. Indirect measurement of blood pressure

The systolic blood pressure was measured according to [3]. In order to determine baseline values, blood pressure values were recorded at least three times on different days for each rat. After recording

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**Table 2**

Plasma ACE concentration at the end of the 3 h treatment. Values are presented as mean ± SEM.

| Treatment group | Rat | OD_{450} (nm) | μg/ml | Average | SD | SEM |
|-----------------|-----|--------------|-------|---------|----|-----|
| GWW             | 1   | 0.592        | 0.14  | 0.15\(^a\) | 0.07 | 0.03 |
|                 | 2   | 0.579        | 0.14  |          |     |     |
|                 | 3   | 0.761        | 0.03  |          |     |     |
|                 | 4   | 0.464        | 0.24  |          |     |     |
|                 | 5   | 0.536        | 0.18  |          |     |     |
| GA              | 1   | 0.181        | 0.80  | 2.08\(^b\) | 0.84 | 0.42 |
|                 | 2   | 0.081        | 2.50  |          |     |     |
|                 | 3   | 0.077        | 2.50  |          |     |     |
|                 | 4   | 0.076        | 2.50  |          |     |     |
| GC              | 1   | 0.089        | 2.50  | 2.50\(^b\) | 0   | 0   |
|                 | 2   | 0.078        | 2.50  |          |     |     |
|                 | 3   | 0.09         | 2.50  |          |     |     |
| GAPI            | 1   | 0.134        | 1.16  | 1.35\(^b\) | 0.52 | 0.3  |
|                 | 2   | 0.11         | 1.94  |          |     |     |
|                 | 3   | 0.156        | 0.95  |          |     |     |
| GDA             | 1   | 0.184        | 0.79  | 1.16\(^b\) | 0.88 | 0.39 |
|                 | 2   | 0.116        | 1.55  |          |     |     |
|                 | 3   | 0.095        | 2.50  |          |     |     |
|                 | 4   | 0.438        | 0.27  |          |     |     |
|                 | 5   | 0.215        | 0.67  |          |     |     |
| GVIKP           | 1   | 0.151        | 0.99  | 0.87\(^b\) | 0.64 | 0.32 |
|                 | 2   | 0.227        | 0.63  |          |     |     |
|                 | 3   | 0.539        | 0.18  |          |     |     |
|                 | 4   | 0.113        | 1.70  |          |     |     |
| GE              | 1   | 0.056        | 2.50  | 2.50\(^b\) | 0   | 0   |
|                 | 2   | 0.057        | 2.50  |          |     |     |
|                 | 3   | 0.057        | 2.50  |          |     |     |
|                 | 4   | 0.056        | 2.50  |          |     |     |
|                 | 5   | 0.062        | 2.50  |          |     |     |
|                 | 6   | 0.057        | 2.50  |          |     |     |
|                 | 7   | 0.058        | 2.50  |          |     |     |
| GE+VIKP         | 1   | 0.062        | 2.50  | 2.29\(^b\) | 0.46 | 0.2  |
|                 | 2   | 0.055        | 2.50  |          |     |     |
|                 | 3   | 0.119        | 1.45  |          |     |     |
|                 | 4   | 0.06         | 2.50  |          |     |     |
|                 | 5   | 0.057        | 2.50  |          |     |     |
the last baseline blood pressure value, an aqueous suspension of each sample was administered to each animal. Three hours after the administration, blood pressure values were recorded with a tail cuff and a pulse sensor (NarcoBiosystems, Houston, TX).

### 2.2.2. Determination of plasma ACE and renin concentrations

A commercial ELISA kit (Rat Angiotensin converting enzyme MBS703086, MyBioSource, CA, USA) was employed to determine ACE concentration according to manufacturer’s directions. This immunoassay is based on a competitive inhibition. Briefly, microtitre plates are coated with ACE. Samples and standards are incubated together with an anti-ACE HRP-labeled conjugate to generate the competition. Plasma renin concentration was determined with a commercial ELISA kit (Rat renin ELISA kit MBS041519 MyBioSource) following the manufacturer’s directions. This immunoassay is a direct ELISA, which has an analytical measurement range of 6.25–200 pg/ml. The final colour reaction was read in a microtiter plate reader (Biotek Synergy HT, Winooski, VT, USA) at 450 nm.

### 2.2.3. Determination of plasma ACE activities

ACE-inhibitory activity was assayed according to [2]. Briefly, to determine the enzymatic activity, 50 μl of buffer (0.2M sodium borate pH 8.3; 2M NaCl), 25 μl of milli Q water, 25 μl of the commercial enzyme (maximum activity control), or plasma samples were incubated with 100 μl of synthetic substrate (HHL) at 37 °C for 30 min. The reaction was stopped by heating the mixture over a water bath at 90 °C for 15 min. After cooling, 600 μl of 0.2M potassium pH 8.2 and 515 μl of colour reagent, which reacts with the hippuric acid generated during the enzymatic reaction, were added and stirred

### Table 3

Plasma renin concentration at the end of the 3 h treatment. Values are presented as mean ± SEM.

| Treatment group | Rat | OD_{450} (nm) | pg/ml | Average | SD | SEM |
|-----------------|-----|--------------|-------|---------|----|-----|
| G_W             | 1   | 0.279        | 0.305 | 54.2    | 59.4 | 42.4^a |
|                 | 2   | 0.183        | 0.171 | 35      | 32.6 |     |
|                 | 3   | 0.252        | 0.221 | 48.8    | 42.6 |     |
|                 | 4   | 0.223        | 0.239 | 43      | 46.2 |     |
|                 | 5   | 0.158        | 0.171 | 30      | 32.6 |     |
| G_A             | 1   | 0.199        | 0.175 | 38.2    | 33.4 | 34.2^b |
|                 | 2   | 0.216        | 0.16  | 41.6    | 30.4 |     |
|                 | 3   | 0.166        | 0.158 | 31.6    | 30   |     |
| G_C             | 1   | 0.193        | 0.178 | 37      | 34   | 35.1^a |
|                 | 2   | 0.176        | 0.159 | 33.6    | 30.2 |     |
|                 | 3   | 0.21          | 0.185 | 40.4    | 35.4 |     |
| G_API           | 1   | 0.13         | 0.151 | 24.4    | 28.6 | 34.0^b |
|                 | 2   | 0.228        | 0.222 | 44      | 42.8 |     |
|                 | 3   | 0.137        | 0.177 | 25.8    | 33.8 |     |
|                 | 4   | 0.193        | 0.184 | 37      | 35.2 |     |
| G_ASH           | 1   | 0.157        | 0.155 | 29.8    | 29.4 | 30.7^b |
|                 | 2   | 0.193        | 0.152 | 37      | 28.8 |     |
|                 | 3   | 0.141        | 0.143 | 26.6    | 27   |     |
|                 | 4   | 0.194        | 0.157 | 37.2    | 29.8 |     |
| G_VIKP          | 1   | 0.192        | 0.15  | 36.8    | 28.4 | 34.9^a |
|                 | 2   | 0.221        | 0.17  | 42.6    | 32.4 |     |
|                 | 3   | 0.18          | 0.181 | 34.4    | 34.6 |     |
| G_E             | 1   | 0.207        | 0.167 | 39.8    | 31.8 | 35.1^a |
|                 | 2   | 0.163        | 0.159 | 31      | 30.2 |     |
|                 | 3   | 0.212        | 0.192 | 40.8    | 36.8 |     |
| G_E+VIKP        | 1   | 0.237        | 0.209 | 45.8    | 40.2 | 36.3^a |
|                 | 2   | 0.192        | 0.175 | 36.8    | 33.4 |     |
|                 | 3   | 0.236        | 0.227 | 45.6    | 43.8 |     |
|                 | 4   | 0.124        | 0.115 | 23.2    | 21.4 |     |
vigorously with a vortex and then centrifuged for 10 min at 20 °C and 3000 × g. The absorbance was measured at 382 nm in a spectrophotometer (Beckman DU 650). The reaction blank was obtained by incubating the synthetic substrate with neither the plasma samples nor the enzyme, completing the reaction volume with milli Q water. Reaction blanks without the substrate (HHL was replaced by 100 μl of borate buffer) and containing plasma samples were also included. Controls containing plasma samples and captopril were also assayed.

2.2.4. Ex vivo experiments

During the surgical procedure employed to obtain blood samples, the thoracic aorta was resected and placed in saline solution bubbled with 5% CO₂ and 95% O₂. The adjacent connective tissue was carefully removed avoiding distention of the vessel and damage to the endothelium. The aorta was

Table 4
Plasma ACE activity. These data are expressed as relative to 100% ACE activity to water control group (Cw). Data are presented as mean ± SEM.

| Treatment group | Rats | DO₃₈₀ (nm) | µg enzyme | DO₃₈₀/µg enzyme | Activity (%)/µg enzyme | Average ± SEM |
|-----------------|------|------------|------------|-----------------|------------------------|---------------|
| GW              | 1    | 0.794      | 0.773      | 0.0037          | 214.6                  | 104.5 ± 101.7 | 100.0 ± 4.8   |
|                 | 2    | 0.790      | 0.725      | 0.0037          | 213.4                  | 103.9 ± 95.5  |
|                 | 3    | 0.688      | 0.752      | 0.0037          | 186.3                  | 90.7 ± 99.0   |
|                 | 4    | 0.785      | 0.798      | 0.0037          | 212.2                  | 103.4 ± 105.1 |
|                 | 5    | 0.768      | 0.721      | 0.0037          | 207.7                  | 101.2 ± 95.0  |
| GA              | 1    | 0.845      | 0.771      | 0.0519          | 16.3                   | 7.8 ± 7.1     | 7.3 ± 0.2     |
|                 | 2    | 0.786      | 0.781      | 0.0519          | 15.1                   | 7.3 ± 7.2     |
|                 | 3    | 0.791      | 0.781      | 0.0519          | 15.2                   | 7.3 ± 7.2     |
|                 | 4    | 0.771      | 0.801      | 0.0519          | 14.9                   | 7.1 ± 7.4     |
| GC              | 1    | 0.733      | 0.790      | 0.0625          | 11.7                   | 5.6 ± 6.1     | 5.8 ± 0.2     |
|                 | 2    | 0.764      | 0.785      | 0.0625          | 12.2                   | 5.9 ± 6.0     |
|                 | 3    | 0.710      | 0.756      | 0.0625          | 11.4                   | 5.5 ± 5.8     |
| GAPi            | 1    | 0.723      | –          | 0.0338          | 21.4                   | 10.3 ± 10.7   | 10.7 ± 0.3    |
|                 | 2    | 0.761      | –          | 0.0338          | 22.5                   | 10.8 ± 10.8   |
|                 | 3    | 0.757      | 0.785      | 0.0338          | 22.4                   | 10.8 ± 11.2   |
|                 | 4    | 0.748      | 0.739      | 0.0338          | 22.1                   | 10.6 ± 10.5   |
| GAH             | 1    | 0.747      | 0.756      | 0.0218          | 34.3                   | 16.5 ± 16.7   | 16.9 ± 0.5    |
|                 | 2    | 0.716      | 0.752      | 0.0218          | 32.8                   | 15.8 ± 16.6   |
|                 | 3    | 0.791      | 0.747      | 0.0218          | 36.3                   | 17.5 ± 16.5   |
|                 | 4    | 0.801      | 0.776      | 0.0218          | 36.7                   | 17.7 ± 17.2   |
|                 | 5    | 0.776      | 0.771      | 0.0218          | 35.6                   | 17.2 ± 17.0   |
| GVIKP           | 1    | 0.794      | 0.799      | 0.0218          | 36.4                   | 17.5 ± 17.6   | 17.7 ± 0.5    |
|                 | 2    | 0.809      | 0.799      | 0.0218          | 37.1                   | 17.8 ± 17.6   |
|                 | 3    | 0.744      | 0.819      | 0.0218          | 34.1                   | 16.4 ± 18.1   |
|                 | 4    | 0.804      | 0.799      | 0.0218          | 36.9                   | 17.7 ± 17.6   |
|                 | 5    | 0.840      | 0.814      | 0.0218          | 38.6                   | 18.5 ± 17.9   |
|                 | 6    | 0.824      | 0.809      | 0.0218          | 37.8                   | 18.2 ± 17.8   |
|                 | 7    | 0.799      | 0.785      | 0.0218          | 36.7                   | 17.6 ± 17.3   |
| GE              | 1    | 0.742      | 0.765      | 0.0625          | 11.9                   | 5.7 ± 5.9     | 5.8 ± 0.1     |
|                 | 2    | 0.785      | 0.790      | 0.0625          | 12.6                   | 6.0 ± 6.1     |
|                 | 3    | 0.742      | 0.756      | 0.0625          | 11.9                   | 5.7 ± 5.8     |
|                 | 4    | 0.751      | 0.756      | 0.0625          | 12.0                   | 5.8 ± 5.8     |
|                 | 5    | 0.765      | 0.770      | 0.0625          | 12.2                   | 5.9 ± 5.9     |
|                 | 6    | 0.742      | 0.747      | 0.0625          | 11.9                   | 5.7 ± 5.8     |
|                 | 7    | 0.725      | 0.775      | 0.0625          | 11.6                   | 5.6 ± 6.0     |
| GE-VIKP         | 1    | 0.785      | 0.684      | 0.0573          | 13.7                   | 6.6 ± 6.7     | 6.4 ± 0.3     |
|                 | 2    | 0.770      | 0.785      | 0.0573          | 13.4                   | 6.5 ± 6.6     |
|                 | 3    | 0.775      | 0.761      | 0.0573          | 13.5                   | 6.5 ± 6.4     |
|                 | 4    | 0.756      | 0.765      | 0.0573          | 13.2                   | 6.4 ± 6.4     |
|                 | 5    | 0.821      | 0.765      | 0.0573          | 14.3                   | 6.9 ± 6.4     |
Table 5
Effect of different samples on isolated aortic rings contracted by exposure to a high concentration of: Potassium ion (80 mM) and Norepinephrine (10⁻⁶ M). Fb1 (g): basal force before K addition. Fb2 (g): basal force before N addition. Fc (g): contractile force. N: norepinephrine. K: potassium ion. AW: aorta weight. Data are presented as mean ± SEM.

| Treatment group | Rats | Fb1 (g) | Fb2 (g) | Fc (g) | AW (mg) | Fc K (g/mg) | Fc N (g/mg) |
|-----------------|------|---------|---------|--------|---------|-------------|-------------|
| Gw              | 1    | 0       | 1.15    | 0.18   | 2.06    | 2.69        | 0.428       | 0.699       |
|                 | 2    | 0.07    | 1.28    | 0.22   | 1.79    | 2.33        | 0.519       | 0.674       |
|                 | 3    | −0.08   | 0.8     | 0.14   | 1.2     | 2.24        | 0.393       | 0.473       |
|                 | 4    | −0.13   | 1.08    | 0.12   | 1.25    | 3.4         | 0.356       | 0.332       |
|                 | 5    | −0.03   | 1.18    | 0.02   | 1.09    | 3.21        | 0.377       | 0.333       |
|                 | 6    | 0.02    | 1.2     | 0.14   | 1.36    | 2.58        | 0.457       | 0.473       |
| average         |      | 0.42a   | 0.50a   |        |         |             |             |
| SD              |      | 0.06    | 0.17    |        |         |             |             |
| SEM             |      | 0.02    | 0.07    |        |         |             |             |
| GA              | 1    | −0.042  | 1.113   | 0.056  | 1.253   | 3.47        | 0.333       | 0.345       |
|                 | 2    | −0.07   | 1.015   | −0.007 | 1.239   | 2.00        | 0.543       | 0.623       |
|                 | 3    | 0.021   | 1.113   | 0.0035 | 1.253   | 2.42        | 0.451       | 0.516       |
|                 | 4    | 0.021   | 0.742   | 0.049  | 1.001   | 1.95        | 0.370       | 0.538       |
| average         |      | 0.44a   | 0.49a   |        |         |             |             |
| SD              |      | 0.11    | 0.14    |        |         |             |             |
| SEM             |      | 0.05    | 0.07    |        |         |             |             |
| GC              | 1    | 0.03    | 1.68    | −0.15  | 1.29    | 3.95        | 0.418       | 0.365       |
|                 | 2    | 0.07    | 1.54    | 0.59   | 1.85    | 3.45        | 0.426       | 0.365       |
|                 | 3    | −0.05   | 1.34    | 0      | 1.57    | 3.12        | 0.446       | 0.503       |
| average         |      | 0.43a   | 0.41a   |        |         |             |             |
| SD              |      | 0.01    | 0.08    |        |         |             |             |
| SEM             |      | 0.01    | 0.05    |        |         |             |             |
| GAPI            | 1    | 2.1     | 2.95    | 2.1    | 3.48    | 2.56        | 0.332       | 0.539       |
|                 | 2    | 1.98    | 2.67    | 2.19   | 3.21    | 3.12        | 0.221       | 0.327       |
|                 | 3    | 2.08    | 3.29    | 2.29   | 3.73    | 3.94        | 0.307       | 0.365       |
|                 | 4    | 0.1     | 0.9     | 0.3    | 1.2     | 2.85        | 0.281       | 0.316       |
|                 | 5    | 0       | 0.9     | 0.1    | 1.1     | 1.9         | 0.474       | 0.526       |
|                 | 6    | 0       | 1       | 0.2    | 1.3     | 2.63        | 0.380       | 0.418       |
| average         |      | 0.332b  | 0.415a  |        |         |             |             |
| SD              |      | 0.087   | 0.098   |        |         |             |             |
| SEM             |      | 0.036   | 0.040   |        |         |             |             |
| GAH             | 1    | −0.1    | 1.2     | 0.4    | 1.5     | 2.50        | 0.52        | 0.44        |
|                 | 2    | −0.1    | 1.5     | 0.3    | 1.8     | 2.98        | 0.537       | 0.503       |
|                 | 3    | 0       | 0.9     | 0.2    | 1.1     | 2.38        | 0.378       | 0.378       |
|                 | 4    | −0.02   | 0.5995  | −0.275 | 0.8085  | 2.06        | 0.301       | 0.526       |
|                 | 5    | 0.06    | 0.6655  | −0.06  | 0.9075  | 1.90        | 0.319       | 0.509       |
|                 | 6    | −0.04   | 0.8525  | −0.099 | 1.089   | 1.91        | 0.467       | 0.622       |
|                 | 7    | 0.07    | 0.528   | −0.165 | 0.759   | 2.24        | 0.204       | 0.413       |
|                 | 8    | 0.03    | 0.7645  | −0.0495| 0.935   | 2.20        | 0.334       | 0.448       |
|                 | 9    | −0.04   | 0.72    | −0.126 | 0.894   | 2.22        | 0.342       | 0.459       |
|                 | 10   | −0.01   | 0.8262  | 0.0108 | 10.476  | 2.41        | 0.347       | 0.430       |
|                 | 11   | 0.04    | 0.624   | 0.042  | 0.72    | 2.01        | 0.291       | 0.337       |
|                 | 12   | −0.07   | 0.402   | −0.042 | 0.528   | 1.87        | 0.252       | 0.305       |
|                 | 13   | 0.02    | 0.520   | 0.016  | 0.668   | 3.28        | 0.152       | 0.199       |
| average         |      | 0.340b  | 0.43a   |        |         |             |             |
| SD              |      | 0.11    | 0.11    |        |         |             |             |
| SEM             |      | 0.03    | 0.03    |        |         |             |             |
| GVIRP           | 1    | 0.014   | 0.868   | 0.049  | 1.316   | 3.15        | 0.271       | 0.402       |
|                 | 2    | 0.077   | 0.861   | 0.077  | 1.239   | 3.00        | 0.261       | 0.387       |
|                 | 3    | −0.098  | 0.84    | −0.049 | 1.204   | 3.30        | 0.284       | 0.389       |
|                 | 4    | 0.014   | 1.162   | 0.07   | 1.61    | 2.86        | 0.401       | 0.538       |
|                 | 5    | 0.02    | 1.27    | −0.15  | 0.84    | 2.75        | 0.455       | 0.360       |
|                 | 6    | 0       | 1.056   | 0      | 1.04    | 2.81        | 0.376       | 0.370       |
|                 | 7    | 0       | 1.24    | −0.016 | 1.1     | 3.37        | 0.368       | 0.331       |

(continued on next page)
then cut into 2 mm long rings. Assay was performed according to [3]. The rings were gently suspended between two stainless steel wires in a water-jacketed organ baths kept at 37 °C and filled with saline solution, bubbled with a mixture of 5% CO2 and 95% O2, giving a pH of 7.40. The lower wire was fixed to a vertical plastic rod immersed in the organ bath, while the upper one was rigidly connected to a force transducer (Grass FT.03D, Grass Telefactor, West Warwick, CT, USA). Preparations were then stretched to obtain a passive force of 2 g and stabilized during 1 h, changing the solution in the chamber every 20 min. Tissue rings were then exposed to a solution containing 80 mM potassium or norepinephrine 10−6 M. For each condition, the contractile response was recorded. At the end of the experiment, tissue rings were dried on filter paper and weighed on a precision scale. The contraction intensity was calculated as the quotient between strength and the weight of the ring (mgF/mg).

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dib.2020.105168.

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