RESPONSE OF IMMUNOREACTIVE ANTIARRHYTHMIC PEPTIDE (IR-AAP) LEVEL ASSOCIATED WITH EXPERIMENTAL ARRHYTHMIA IN RATS

YASUHIRO KOHAMA, KAZUHISA IWABUCHI, TAKEHI SHIBAHARA, MASARU OKABE AND TSUTOMU MIMURA

Faculty of Pharmaceutical Sciences, Osaka University, Yamadaoka 1-6, Suita, Osaka, 565, Japan

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The change in endogenous antiarrhythmic peptide (AAP) levels in serum, heart and kidney from rats under several drug-induced arrhythmias was investigated using a sensitive and specific radioimmunoassay. The extracts from serum, heart and kidney were fractionated by Sephadex G-25 chromatography to obtain a fraction which was found at the same position as that of synthetic AAP. In serum, the immunoreactive (IR)-AAP level increased about threefold under CaCl$_2$-, aconitine- and epinephrine-induced arrhythmias. In heart, the IR-AAP level was doubled by CaCl$_2$ increased 1.4 times by aconitine and decreased by one third by epinephrine. The levels in serum and heart were slightly increased by ADP. The kidney IR-AAP level was not changed under these drug-induced arrhythmias. Considering the previous result that AAP could protect against CaCl$_2$- and aconitine-induced arrhythmias but not against epinephrine-induced arrhythmia, the change in the IR-AAP level in heart coincided with the effect of AAP given to animals under arrhythmia. Quinidine, propranolol and verapamil had no effect on serum IR-AAP level. These results suggested that endogenous AAP in heart worked to suppress certain arrhythmia.

**Keywords** — peptide; radioimmunoassay; arrhythmia; rat; serum; heart

A hexapeptide (antiarrhythmic peptide, AAP), isolated from bovine atria$^1$ and identified as Gly-Pro-Hyp-Gly-Ala-Gly,$^9$ has a protective effect against experimental drug-induced heart arrhythmias via the improvement of permeability of ions through the myocardial cell membrane.$^8$ The peptide was also effective in various thrombosis models$^4$ and its platelet antiaggregant action was demonstrated to be a cause of the antithrombotic effect.$^5$ In our preceding report, a sensitive and specific radioimmunoassay for AAP was described and validated for measuring immunoreactive (IR)-AAP in rats.$^6$ The highest level of IR-AAP was found in heart, followed by kidney, with the levels in serum and other tissues less than 2% of that in heart.$^6$

In this paper, data suggesting the physiological role of endogenous AAP have been provided for the first time by demonstrating the change of IR-AAP levels in serum, heart and kidney of rats under experimental arrhythmia.

**MATERIALS AND METHODS**

*Materials and Animals* — Aconitine, propranolol hydrochloride, verapamil hydrochloride and epinephrine bitartrate were products of Sigma Chemical Co., St. Louis MO. Adenosine 5’-diphosphate disodium salt (ADP) was obtained from Kohjin Co., Ltd., and quinidine sulfate from Wako Pure Chem. Ind., Ltd. The reagents for radioimmunoassay were obtained as described previously.$^6$ Eight to 13-week-old Wistar male rats were anesthetized with pentobarbital (40 mg/kg, i.p.) for injection or infusion of sample, removal of tissues and blood collection from the abdominal artery.

*Experimental Arrhythmia* — Arrhythmia models were made by giving CaCl$_2$, aconitine, ADP, or epinephrine to rats as described previously.$^3$ Briefly, CaCl$_2$ (100 mg/kg), aconitine (10 µg/kg) or epinephrine (140 µg/kg) was injected into the femoral vein. Developments of ectopic beat, A-V block and/or ventricular fibrillation on ECG (lead II) were monitored using a polygraph system (Nihon Koden RM-6000) immediately after the injection. Saline was used as a control. ADP (1 mg/kg) was infused at 0.05 ml/min/330 g body weight into the femoral vein and developments of T wave inversion, ST segment depression and ectopic beat on ECG (lead I) were monitored.

*Extraction of Tissues and Serum* — The blood, heart and kidney were removed from the rats 3 to 5 min after injection of saline, CaCl$_2$,
aconitine or epinephrine, and 5 min after the start of ADP infusion. At these time periods, all rats except the control were under arrhythmia. The blood was also removed from rats 3 to 5 min after intravenous injection of quinidine (10 mg/kg), propranolol (2.5 mg/kg) or verapamil (0.5 mg/kg). All tissues and serum separated from blood were extracted using a hot procedure as described previously.6) The organ parts were minced, homogenized in 0.05 N acetic acid, autoclaved and centrifuged. The supernatant fluid was lyophilized and the extract dissolved in 2.5 mM EDTA (pH 8.0) for assay. Part of the extract was fractionated on a Sephadex G-25 column which was calibrated with synthetic AAP, and the fractions were also assayed for IR-AAP content.

Radioimmunoassay — IR-AAP in extracts and fractions was determined using the method described in the preceding paper.6) Briefly, the procedure was as follows. One hundred μl of 125I-HPP-AAP (ca. 10000 cpm), 100 μl of guinea pig anti-bovine serum albumin (BSA)-AAP serum (final dilution; 1/800), 200 μl of sample or standard synthetic AAP solution and 400 μl of assay buffer were mixed and incubated at 4 °C for 2 d. The assay buffer (diluent) was phosphate-buffered saline (pH 7.5)–25 mM EDTA supplemented with 0.5% BSA and 0.01% merthiolate. The polyethylene glycol method was employed to separate free AAP from antibody-bound AAP and the B/F ratio was calculated.

RESULTS

CaCl2-Induced Arrhythmia

The extracts obtained from serum, heart and kidney of control and CaCl2-treated rats were fractionated with Sephadex G-25, and their IR-AAP concentration profiles are shown in Figs. 1, 2 and 3. In serum, all of the IR-AAP was derived from Fr. II, which was found at the same position as that of AAP. In heart and kidney, the IR-AAP was separated into Fr. I, which emerged at the position of void volume, and Fr. II. In the control rats, immunoreactivity of heart Fr. II was about 50% of the total IR-AAP in the extracts but only about 10% in kidney. The Fr. II

FIG. 1. Gel Filtration of Serum Extracts from Control and CaCl2-Treated Rats on Sephadex G-25 Column

A one ml aliquot of extracts (2 ml serum/ml) was applied on the column (1.2×160 cm) which was washed with distilled water.
a) Peak volume for synthetic AAP.
○, control rat; ●, CaCl2-treated rat.

FIG. 2. Gel Filtration of Heart Extracts from Control and CaCl2-Treated Rats on Sephadex G-25 Column

A one ml aliquot of extracts (1 g heart/ml) was applied on the column (1.2×160 cm) which was washed with distilled water.
a) Peak volume for synthetic AAP.
○, control rat; ●, CaCl2-treated rat.
peak markedly increased in serum and heart extracts of CaCl₂-treated rats, but peaks of heart Fr. I, kidney Fr. I and Fr. II were not different from those of the control. The changes of IR-AAP levels in serum, heart and kidney by CaCl₂-treatment are summarized in Table I. Total IR-AAP levels in serum and heart significantly increased but the level was unchanged in kidney. In heart, the increase was due to that of Fr. II, which was double the control level. Both total IR-AAP and Fr. II levels of kidney were the same as those of the control.

**Aconitine-, ADP- and Epinephrine-Induced Arrhythmias**

The extracts obtained from heart and kidney of aconitine-, ADP- and epinephrine-treated rats were fractionated with Sephadex G-25 to give Fr. II (Fr. No. 30–34) as mentioned above. The serum extract and Fr. II from tissues were assayed for immunoreactivity. The results are shown in Table II. The level of serum IR-AAP significantly increased in both aconitine-and epinephrine-treated rats, about threefold, and

**TABLE I. IR-AAP Levels of Serum, Heart and Kidney in CaCl₂-Treated Rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum IR-AAP (pmol/ml or g, mean ± s.e., n = 5)</th>
<th>Heart IR-AAP (pmol/ml or g, mean ± s.e., n = 5)</th>
<th>Kidney IR-AAP (pmol/ml or g, mean ± s.e., n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Fr. II</td>
<td>Total</td>
</tr>
<tr>
<td>Control</td>
<td>2.3 ± 0.7</td>
<td>203 ± 14</td>
<td>98 ± 7</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>7.2 ± 0.8</td>
<td>317 ± 14</td>
<td>212 ± 9</td>
</tr>
</tbody>
</table>

(213) 

(56) 

(-9) 

(-6)

*a* Change % from control was given in parenthesis.

*b* p < 0.001: versus control.

**TABLE II. IR-AAP Levels of Serum, Heart and Kidney in Aconitine, ADP or Epinephrine-Treated Rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum IR-AAP (pmol/ml or g, mean ± s.e., n = 5)</th>
<th>Heart IR-AAP (pmol/ml or g, mean ± s.e., n = 5)</th>
<th>Kidney IR-AAP (pmol/ml or g, mean ± s.e., n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Fr. II</td>
<td>Total</td>
</tr>
<tr>
<td>Control</td>
<td>2.3 ± 0.7</td>
<td>98 ± 7</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>Aconitine</td>
<td>6.4 ± 0.7</td>
<td>141 ± 10 <em>c</em></td>
<td>14 ± 2</td>
</tr>
<tr>
<td>ADP</td>
<td>3.6 ± 1.6</td>
<td>116 ± 6</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>7.5 ± 1.5</td>
<td>29 ± 6 <em>d</em></td>
<td>19 ± 3</td>
</tr>
</tbody>
</table>

(178) 

(44) 

(18) 

(12)

(57) 

(18) 

(12)

(226) 

(-90) 

(12)

*a* Change % from control was given in parenthesis.

*b* p < 0.05, *c* p < 0.01, *d* p < 0.001: versus control.
Change of IR-AAP in Rats

TABLE III. Effect of Antiarrhythmic Drugs on Serum IR-AAP Level in Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, i.v.)</th>
<th>IR-AAP (pmol/ml, mean ± s.e., n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>2.3 ± 0.7</td>
</tr>
<tr>
<td>Quinidine</td>
<td>10</td>
<td>2.0 ± 0.9 (−13) a)</td>
</tr>
<tr>
<td>Propranolol</td>
<td>2.5</td>
<td>2.1 ± 0.9 (−9)</td>
</tr>
<tr>
<td>Verapamil</td>
<td>0.5</td>
<td>2.2 ± 0.4 (−4)</td>
</tr>
</tbody>
</table>

a) Change % from the control group was given in parenthesis.

was increased by 57% in ADP-treated rats, which was not significant. Heart Fr. II level was significantly increased by 44% in aconitine-treated rats, significantly decreased by 70% in epinephrine-treated rats, and did not change in ADP-treated rats. Kidney Fr. II was at the control level in all groups. Quinidine, Propranolol and Verapamil Treatments

IR-AAP levels in the extracts obtained from serum of quinidine-, propranolol- and verapamil-treated rats were determined. These drugs had no effect on serum IR-AAP level, as shown in Table III.

DISCUSSION

The IR-AAP in heart and kidney of normal rats was separated into Fr. I and Fr. II by Sephadex G-25 chromatography, whereas the IR-AAP in serum was found only in Fr. II. It was previously reported that Fr. I was a large molecular size substance such as an AAP-precursor or an AAP-protein complex and that Fr. II contained AAP itself. In this experiment, IR-AAP in CaCl<sub>2</sub>-treated rats consisted of the two fractions in heart and kidney, and only Fr. II in serum, as well as in the control rats. The change of IR-AAP level occurred only in Fr. II, as shown in both serum and heart of CaCl<sub>2</sub>-treated rats. In order to evaluate the physiological role of AAP under arrhythmia, the level of Fr. II containing AAP (which itself possesses antiarrhythmic ability) was estimated.

The changes of Fr. II levels have been demonstrated in rats under several drug-induced arrhythmias. In serum, the IR-AAP level increased about threefold under CaCl<sub>2</sub>-, aconitine- and epinephrine-induced arrhythmias. In heart, in which Fr. II is present in the highest concentration and exhibits its action, the changes of the level under these conditions were different from those of serum. The heart Fr. II level was doubled by CaCl<sub>2</sub>, increased by 1.4 by aconitine and decreased by 1/3 by epinephrine. The levels in serum and heart were slightly increased by ADP. The kidney Fr. II level did not change under these drug-induced arrhythmias. Thus, serum and heart Fr. II levels were responsive to arrhythmia. The increases in serum level is probably due to release or efflux of heart Fr. II. The change in heart Fr. II level is considered to be the result of the changes in production, accumulation, release and/or efflux of Fr. II. It is generally thought that excess CaCl<sub>2</sub> causes arrhythmia mainly via Ca-induced Ca-release from a sarcoplasmic reticulum and generation of a transient inward current;<sup>7</sup> aconitine via increase of Na inward current;<sup>8</sup> epinephrine via increase of Ca influx via induced cyclic AMP by a β-adrenergic effect;<sup>9</sup> and ADP via ischemia by a platelet aggregation.<sup>10</sup> It appears that the action mechanisms of CaCl<sub>2</sub>, aconitine and epinephrine are closely related to production and/or movement of Fr. II in the heart, as the change of heart Fr. II level was slight in the ADP-induced arrhythmia which occurred via ischemia. At present, however, the mechanism of change in the Fr. II level is not clear. Previously, we reported that exogenous AAP significantly reversed the persistent arrhythmias consisting of A-V block, ectopic beat and/or ventricular tachycardia induced by aconitine treatment to normal synus rhythm, and significantly prolonged the onset time of A-V block or ectopic beat induced by CaCl<sub>2</sub>-infusion, but did not affect epinephrine-induced arrhythmia.<sup>30</sup> The effectiveness or ineffectiveness of AAP given to animals under arrhythmia coincided with the increase or decrease of heart Fr. II level in the present study. It is highly probable that endogenous AAP in the heart works to suppress arrhythmias.
induced by CaCl₂ and aconitine. One interpretation of the data, in the case of epinephrine-induced arrhythmia, is that the β-adrenergic mechanism in the heart may work to exhaust endogenous AAP and to exclude exogenous AAP, but this requires confirmation. In any event, the Fr. II level was largely changed in the serum and heart of rats under CaCl₂⁻, aconitine- and epinephrine-induced arrhythmias, strongly suggesting a physiological significance of AAP.

Quinidine, propranolol and velapamol are well known antiarrhythmic drugs which have different action mechanisms. Generally, the normal animals without arrhythmia respond to the actions of these drugs. We tested the effect of these drugs on the serum IR-AAP level which was most responsive to arrhythmia. The IR-AAP level remained unchanged, however, suggesting that endogenous AAP was not associated with the effects of these antiarrhythmic drugs.

REFERENCES