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Cardioactive effects from freshwater algae *Oscillatoria limnetica*, *Hydrodictyon reticulatum*, and *Microcoleus lacustris*

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ABSTRACT - The cardiac activity of the hexane, chloroform, methanol and water extracts from *O. limnetica*, *H. reticulatum* and *M. lacustris* were investigated in isolated heart preparations in Wistar rats. The bolus injections of hexane extract from *O. limnetica*, water extract of *H. reticulatum* and chloroform extract from *M. lacustris* produced significant coronary dilator action and a protective effect against reperfusion-arrhythmias in the isolated and perfused rat heart. *M. lacustris* is more active than *O. limnetica* and *H. reticulatum*. These algae possess significant antianginal and antiarrhythmic properties.

KEYWORDS - *Oscillatoria limnetica*, *Hydrodictyon reticulatum*, and *Microcoleus lacustris*.

INTRODUCTION

In order to survive in a highly competitive environment, freshwater or marine algae have to develop defense strategies that result in a tremendous diversity of compounds from different metabolic pathways. Recent trends in drug research from natural sources have shown that algae are promising organisms to furnish novel biochemically active compounds (1). Several substances biosynthesized by algae have potential economic impact in food science, pharmaceutical industry and public health. Emphasis is given to fatty acids, steroids, carotenoids, polysaccharides, lectins, mycosporine-like amino acids, halogenated compounds, polyketides and toxins. *Oscillatoria* cf. *limnetica* (Cyanoprocaryota) affects in varying degrees the growth of gram-positive and gram-negative heterotrophic bacteria, as well as of the yeast *Saccharomyces cerevisiae* (2) and the pathogenic species *Staphylococcus aureus*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Candida albicans* as well the bacterial contamination indicators *Escherichia coli* (faecal indicator), *Streptococcus faecalis* (enterococci), *Enterobacter aerogenes* ("coliforms"), *Staphylococcus epidermidis* (dermic germ) and *Bacillus subtilis* (contamination germ). The hexadecenoic acid (C16:1) of aerobically grown *O. limnetica* was shown to contain both the delta-7 (79%) and delta-9 (21%) isomers, while the octadecenoic (C18:1) acid was entirely the delta-9 acid

(3). These compounds were responsible for the antimicrobial activity. Also five diacylated sulfoglycolipids and four acylated diglycolipids were isolated from the cyanobacterium *O. limnetica* (TAU strain NG-4-1-2), were found to inhibit HIV-1 RT enzymatic activity to different extents (4). It was found that *Hydrodictyon reticulatum* exert a strong antibacterial effect (5). Antibacterial substances (unsaturated fatty acids with 16 or 18 C-atoms) from *H. reticulatum*, were effective only against grampositive strains (6). The three algae have not been reported yet, studies pharmacology. The present paper reports the cardiac activity of the hexane, chloroform, methanol and water extracts from *O. limnetica*, *H. reticulatum* and *M. lacustris* on the hyperkinetic ventricular arrhythmias by post-ischaemic reperfusion (HVAR) in the isolated and perfused heart .

MATERIALS AND METHODS

Source sample collection

O. limnetica. and *H. reticulatum* field samples were collected from planta de tratamiento, del cerro de la estrella, D.F. in march 2006. *Microcoleus lacustris* field samples were collected from Balsas basin in February and July 2006, from one spring, Los Manantiales, Morelos state and were taxonomically authenticated in the Laboratorio de Investigación de Ficológia de la Facultad de Ciencias UNAM and a

voucher specimen no. of the plant is stored for reference. After the collection, the samples were kept in an ice chest. Upon return to the laboratory, the samples were washed with distilled water and extraneous matter was removed and subsequently used in the test.

Preparation of extract

O. limnetica and *H. reticulatum* samples were dried at room temperature and ground into a fine powder. 50g of powder was heated to reflux temperature (Soxhlet) with 1.5L of hexane, chloroform, methanol and water for 3h. The solvents were removed under reduced pressure using an evaporator to constant weight. The % yields of hexane, chloroform, methanol and water from *O. limnetica* were 2.5, 4.8, 8.5 and 14.0%, *H. reticulatum* 15.3, 10.8, 9.4 and 9.8% and *M. lacustris* respectively. Extracts of algae were sonicated before addition to the organ bath, extracts were prepared by adding the substance directly to Krebs-Henseleit solution.

Biological experimental procedures

Animals

Wistar rats (250 to 300 g) were used for all experiments. The animals were housed in a cage under conditions of standard light (light on from 7.0 a.m. to 7.0 p.m.), temperature ($22 \pm 1^\circ\text{C}$) and room humidity ($60 \pm 10\%$) conditions for one week before the experimental sessions. The animals were given a commercial feed prepared by Purina and allowed tap water *ad libitum*. The procedures involving animals and their care conformed to the international guidelines Principles of Laboratory Animals Care.

Tissue preparation

Isolated and perfused rat heart. Hearts from rats were perfused by Langendorff technique as previously described (7) with some modifications. Rats, were anaesthetized with urethane (1.2 g/kg, i.p.), 500 UI of heparin (Vister, Parke-Davis) was injected intraperitoneally and the animals were killed by exsanguination. The heart was carefully dissected freed of adhering tissue from its connections and mounted intact on the Langendorff heart apparatus and perfused retrogradely via the aorta with a modified Krebs-Henseleit solution of the following composition (nM): NaCl 118.0, KCl 4.7, MgSO_4 1.2, KH_2PO_4 1.2, CaCl_2 2.55, NaHCO_3 25.0, glucose 11.0, at a constant pressure of 40 mm Hg, O_2 -aerated and maintained at 37°C .

Data recording - Heart rates were monitored throughout each experiment by electrocardiograms

recorded by micro-electrodes attached to the right atrial appendage and to the right and left ventricular walls of the isolated heart. The electrodes were directed through to an analog-to-digital converter (MacLab) and recorded on a Macintosh computer.

Test extracts

Extracts were administered through a polyethylene cannula in 1 ml/min retrograde perfusions of 1 mM concentration. The freshly prepared solutions were introduced into the system only when the hearts had stabilized after being mounted onto the Langendorff apparatus (usually 30 min). After 30 min stabilization the effects of the obtained extracts were recorder (8). Separated preparations were used for each concentration of the tested agents. Test extracts were added to the bath at concentrations of 1, 2, 3 and 4 $\mu\text{g}/\text{mL}$. Control hearts received vehicle only (0.2 mL of ethanol-saline 1:1 v/v).

Data analysis

At least six experiments were conducted for each treatment. Data were analyzed by paired t-tests with probabilities of less than 0.05 considered statistically significant.

RESULTS

In all groups arrhythmias occurred to varying degrees. Addition of 1, 2, 3 and 4 $\mu\text{g}/\text{mL}$ of hexane extract from *O. limnetica*, water extract of *H. reticulatum* and chloroform extract from *M. lacustris* shortened the duration of ventricular premature beat (VP), ventricular tachycardia (vr), and ventricular fibrillation (VF) after ligation and reperfusion (values are given in Table 1). *M. lacustris* is more active than *O. limnetica* and *H. reticulatum*. Other extracts from algae did not show any activity. Model protective effects on reperfusion-induced hyperkinetic ventricular arrhythmias in the rat isolated heart is considered as a method in which the consequences of post-ischaemic reperfusion are the most similar to the early arrhythmias occurring in man after coronary occlusion-reperfusion (9). In this experimental model ventricular premature beats, ventricular tachycardia and ventricular fibrillation were seen as the most severe types of early arrhythmias. In the control hearts, the incidence of these types of arrhythmias was 80%. All four concentrations of extracts (1, 2, 3 and 4 $\mu\text{g}/\text{mL}$) assayed decreased the incidence of reperfusion-arrhythmias but only at 3-4 $\mu\text{g}/\text{mL}$ concentration also reduced the severity of arrhythmias.

DISCUSSION

Oscillatoria limnetica demonstrated moderate antimicrobial activity against *Saccharomyces*

Table 1. Activity of hexane extract from *O. limnetica* (O.L), water extract of *H. reticulatum* (H.R) and chloroform extract from *M. lacustris* (M.L.) on the hyperkinetic ventricular arrhythmias by post-ischaemic reperfusion (HVAR) in the isolated and perfused heart

Treatment	Dose ($\mu\text{g/mL}$)	HVAR incidence (%)	Severity (degree Lown)
Control		80	4b
O.L	1	80	4b
	2	70	4b
	3	60	3
	4	40	2
H.R	1	80	4b
	2	80	4b
	3	70	4b
	4	50	3
M.L.	1	80	4b
	2	60	4b
	3	40	2
	4	30	2
Verapamil	1	20	2

HVAR classed according to Lown et al., 1975 (10). Degree: 0, no ventricular ectopic beats; 1, occasional isolated, premature ventricular beats (PVB); 2, frequent PVB, minor 30/h; 3, multiform PVB; 4, repetitive PVB (A, couplets; B, salvos); 5, early PVBR/T.

cerevisiae (2), *Staphylococcus aureus*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Candida albicans* *Escherichia coli*, *Streptococcus faecalis*, *Enterobacter aerogenes*, *Staphylococcus epidermidis* and *Bacillus subtilis*. Hexadecenoic acid (C16:1) and octadecenoic (C18:1) acid were responsible for the activity reported for the extracts (3). Diacylated sulfoglycolipids, acylated diglycolipids exhibited activity against HIV-1 (4). *Hydrodictyon reticulatum* showed strong antibacterial activity (5). Unsaturated fatty acids with 16 or 18 C-atoms showed only activity against grampositive microorganism (6). Verapamil work as antiarrhythmic drugs, it is a calcium ion influx inhibitor (calcium entry blocker or calcium ion antagonist). The mechanism of the antianginal and antiarrhythmic effects of verapamil is believed to be related to its specific cellular action of selectively inhibiting transmembrane influx of calcium in cardiac muscle, coronary and systemic arteries and in cells of the intracardiac conduction system. Verapamil blocks the transmembrane influx of calcium through the slow channel (calcium ion antagonism) without affecting, to any significant degree the transmembrane influx of sodium through the fast channel. This results in a reduction of free calcium ions available within cells of the above tissues (10).

The bolus injection of hexane extract from *O. limnetica*, water extract of *H. reticulatum* and chloroform extract from *M. lacustris* produced significant coronary dilator action and a protective effect against reperfusion-arrhythmias in the isolated and perfused rat heart (11). It is known that the calcium-antagonists are effective drugs in the experimental models used in this study (12).

CONCLUSION

The effects of extracts of *O. limnetica*, *H. reticulatum* and *M. lacustris* on rat isolated heart are presented. Also compared the effects of extracts obtained from algae with those of verapamil. It is, the commonly used antiarrhythmic drug exhibited similar activity at the algae. All the concentrations of extracts assayed decreased the incidence of reperfusion-arrhythmias. Mechanism of the antiarrhythmic effect of the extracts remains to be clarified. Fractionation of the extracts is in progress to identify the active fractions, isolate and characterize the active compounds.

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