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EFFECTS OF OSCILLATORIA AGARDHII-TOXINS ON BLOOD PRESSURE AND ISOLATED ORGAN PREPARATIONS

By

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BERG, KJETIL and NILS EIVIND SØLI: *Effects of Oscillatoria agardhii-toxins on blood pressure and isolated organ preparations.* Acta vet. scand. 1985, *26*, 374—384. — Toxic extracts of the blue-green alga Oscillatoria agardhii were tested for their effect on various isolated laboratory animal preparations. The Oscillatoria-toxins did not show hemagglutinative or hemolytical properties. An immediate rise in blood pressure was seen after intravenous injection in rats. As regards isolated organs, effects were most marked on the liver. Addition of toxic extract to isolated liver perfusion fluid led to physiological, biochemical and pathological changes indicating severe damage to the organ. Negative chronotropic and inotropic effects were found on the isolated heart. The toxins did not influence isolated smooth muscle or nerve striated muscle preparations.

blue-green algae; hepatotoxins.

Increasing attention is being paid worldwide to problems associated with toxin-producing blue-green algae. Blooms of these algae may form in eutrophic waters, and present hazards both to animal and human health (*Skulberg et al.* 1984). Few of the toxins in question have as yet been identified, but they are generally classified in two groups, peptides and alkaloids. Their effects are considered to be hepatotoxic and neurotoxic, respectively (*Carmichael* 1982, *Codd* 1984).

In a study on mice and rats, the blue-green alga Oscillatoria agardhii was found to produce toxins with mainly hepatotoxic effects (*Berg & Søli* 1985). This paper reports the results of various toxicological experiments with extracts of the organism. The tests were performed on isolated laboratory animal preparations, in order to further clarify the main mechanisms of action

of the toxins. It was also of interest to compare the results obtained with findings reported with other hepatotoxic blue-green algae.

MATERIAL AND METHODS

Freeze-dried material of Oscillatoria agardhii was used in the experiments. The algae originated from 2 eutrophic lakes, lake Gjersjøen and lake Årungen, located in close proximity in Akershus, Norway. Material from water blooms in both lakes was collected in 1981. Previous acute toxicity tests on mice and rats had revealed that extracts of the lake Gjersjøen material were toxic, with an approximate MLD_{100} intraperitoneal of 200 mg/kg, while extracts of the lake Årungen material were non-toxic.

Preparation of extracts

Extracts were made in sterile 0.9 % NaCl, equivalent to 50 mg freeze-dried algal material per ml. The extraction period was 30 min at 20°C, with regular stirring. The extracts were then centrifuged for 10 min at 4000 g and the supernatants used for further experiments. For the lake Gjersjøen extract, this represented a toxin concentration of 12.5 mouse units per ml, a mouse unit being defined as the minimum dose necessary to kill a 20 g mouse within 4 h.

Hemagglutination test

Equal volumes (0.1 ml) of algal extracts and a 1 % suspension of erythrocytes (washed 3 times in sterile 0.9 % NaCl) were mixed in microtiter V-plates. Parallels were incubated at 20°C and 37°C for 5 h. The plates were checked every 60 min. Series of extract dilutions (undiluted 50 g/ml, 1:2, 1:4 and 1:10), were tested on various types of erythrocytes (mouse, rat, sheep, goat, cattle and human). Lack of formation of an erythrocyte pellet was considered to constitute a positive hemagglutination reaction.

Hemolysis test

3.0 ml of rat, sheep, goat and cattle blood in tubes were mixed with various amounts of the 50 mg/ml test extracts (extract volumes 50, 10 and 1 % of the total mixture volum), and incubated in a water bath at 37°C. Samples were collected every 30 min for 5 h, centrifuged, and the plasma hemoglobin concentrations measured spectrophotometrically.

Blood pressure study

Test animals (rats, Mol: WIST, males, 300—350 g), were anaesthesized with urethanum (1.0 ml 12.5 % solution per 100 g body weight given intraperitoneally). Polyethylene catheters were introduced into the jugular vein and the carotid artery, and the catheters fixed into position by means of ligatures. Filtered extracts (membrane filters, pore size 0.45 μ) were administered through the jugular vein catheter, the volume standardized to 1.0 ml given during a period of 25 s. Extract concentrations of 50 and 25 mg/ml were tested. Blood pressure was measured by connecting the carotid artery catheter to a pressure transducer coupled to a recorder and a 10 speed chart mover (Harvard Apparatus Co., 150 Dover Road, Millis, Mass. 02054, USA).

Isolated organ studies

Effects of the blue-green algal extracts were examined on a number of isolated organs. These studies were carried out as in vitro tests, mainly on rat ogans. The various organs were dissected free, removed from the body and assembled in suitable organ perfusion baths. The algal extracts were added directly to the perfusion liquids, and effects on normal organ characteristics observed and measured. Four to 6 organs were used in each type of experiment.

Isolated liver preparation

Male rats were used as liver and blood donors. The animals were anaesthesized with methoxyflurane (inhalation anaesthesia). The experimental procedure used was described by *Seglen & Jervell* (1969). Following laparatomy, the portal vein and the bile duct were cannulated with polyethylene catheters, and the catheters fixed with ligatures. The liver was then dissected free and moved to a special perfusion system. During the surgery, the organ was supplied with oxygenated perfusate via the portal vein catheter.

Each liver was perfused with 30 ml of 33 % rat blood prepared by mixing heparinized, freshly drawn blood with 2 parts of Krebs-Ringer bicarbonate solution (pH 7.4) containing 2.5 g bovine albumin per 100 ml perfusion fluid. The temperature of the perfusion fluid was maintained at 38°C, and the perfusion pressure was 16 cm of water. The perfusate was oxygenated in

a wire mesh oxygenator with a humidified mixture of 0_2 and CO_2 (95:5). Perfusion flow rate was about 6 ml/min. After connection of the liver, the system was allowed 20 min for equilibration. 2.0—4.0 ml of filtered test extract (concentration 50 mg/ml) was then added to the perfusate. Liver temperature, bile excretion and perfusate flow were continuously recorded. Perfusate samples for biochemical analysis were collected at 0, 60 and 120 min.

Perfusate parameter assays

Plasma concentrations of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (AP), gamma-glutamyl transpeptidase (γ-GT), glucose and bile acids were measured. ASAT, ALAT, AP and γ-GT values were measured on an automatic Gemsaec fast analyser (reagents from J. T. Baker Chemicals B.V., P. O. Box 1, Deventer, Holland). Glucose was measured on a Seralyzer® Reflectance Photometer using Seralyzer® Glucose Reagent Strips (Ames Division, Miles Laboratories, Inc., P. O. Box 70, Elkhart, Ind. 46515, USA), and bile acids on a spectrophotometer using an Enzabile® kit (Diagnostics Division, Nyegaard & Co., P. O. Box 4220 Torshov, 0401 Oslo 4, Norway). Histological examinations were performed on the perfused livers.

Isolated heart preparation

Rat hearts were dissected free and cannulated through the aorta. The cannula was fixed with a ligature, and connected to a perfusion apparatus. Oxygenated Krebs-Ringer solution was used as perfusate, and the temperature kept at 38°C. To record contractions of the organ, the apex of the heart was connected to a heart/smooth muscle transducer with a thread, and further coupled to a recorder and a 10 speed chart mover (Harvard Apparatus Co.). 1.0 ml of the test extract was injected through the perfusion cannula during a period of 25 s (extract concentrations 50 and 25 mg/ml).

Isolated ileum preparation

The terminal ileum from guinea pig (Ola: DH) was used as test organ. The section of intestine was removed from starved animals, and mounted in an organ bath filled with oxygenated Tyrode solution. The temperature was kept at 38°C. Contractions were measured by using the same equipment as in the isolated

heart experiment. 1.0—4.0 ml of test extract (concentration 50 mg/ml) was added directly to the bath solution. Carbachol was used to check the function of the preparation.

Isolated phrenic nerve diaphragm preparation

The experiment was done with organs from rat. The preparation was mounted in an organ bath filled with oxygenated Tyrode solution, and the temperature kept at 38°C. The phrenic nerve was connected to an electric stimulator, and contractions of the diagphragm measured by using the same equipment as in the isolated heart and isolated ileum studies. 1.0—4.0 ml of the test extract (concentration 50 mg/ml) was added directly to the bath solution. Physostigmine and tubocurarine were used to check the function of the preparation.

RESULTS

Effects on hemagglutination

After an incubation period of 60 min, the erythrocytes were all pelleted in the microtiter V-plates. This applied for all dilutions of extracts originating both from lake Gjersjøen and from lake Årungen (final concentrations up to 25 mg/ml). The results were the same at both incubation temperatures used, and the different erythrocytes all responded in the same way.

Effects on hemolysis

Addition of algal extracts in concentrations up to 25 mg per ml sample (50 volume per cent extract) did not give any rise in plasma hemoglobin values. This was true both with material originating from lake Gjersjøen and from lake Årungen. The results were not influenced by the type of blood used.

Effects on blood pressure

Lethal extract concentrations (25—50 mg) of material originating from lake Gjersjøen produced a marked increase in blood pressure, both systolic and diastolic, immediately after injection. The values rose from around 120:80 up to 190:150. Thereafter, there was a gradual decrease until normal values were reached after about 20 min. The systolic and diastolic blood pressure continued to fall evenly until death occurred after 90—

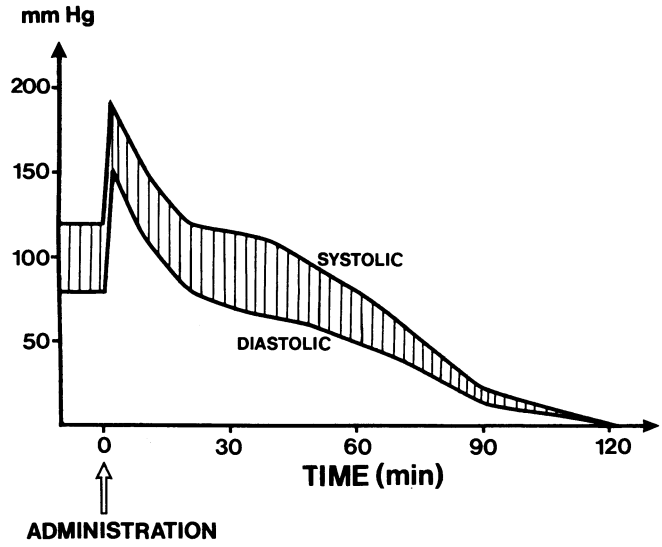

Figure 1. A compressed diagram showing systolic and diastolic blood pressure in an intoxicated rat. Animal injected with 1.0 ml (50 mg/ml) Oscillatoria agardhii-extract from lake Gjersjøen.

120 min (Fig. 1). Extracts of material from lake Årungen did not cause any major changes in the blood pressure during this time period.

Effects on isolated liver preparation

Perfused control livers functioned well for 120 min, after which temperature and perfusate flow slowly started to decline. Bile excretion ceased after 60—90 min. Adding a lethal dose (100—200 mg) of extract of the lake Gjersjøen material rapidly resulted in a complete stop of bile excretion. Temperature and perfusate flow of the isolated liver also instantly declined, and the organ stopped functioning within 90 to 120 min, depending on the dose of extract given (Fig. 2). ASAT and ALAT values were significantly higher than with perfused control livers (Fig. 3), and plasma glucose levels lower. AP, γ-GT or bile acid values were not altered.

Histologically, dissociation and degeneration of hepatocytes were the main findings. Increased amounts of erythrocytes were also present in the sinusoids. Changes were most prominent in the centrolobular areas. Some of the hepatocytes were irregular

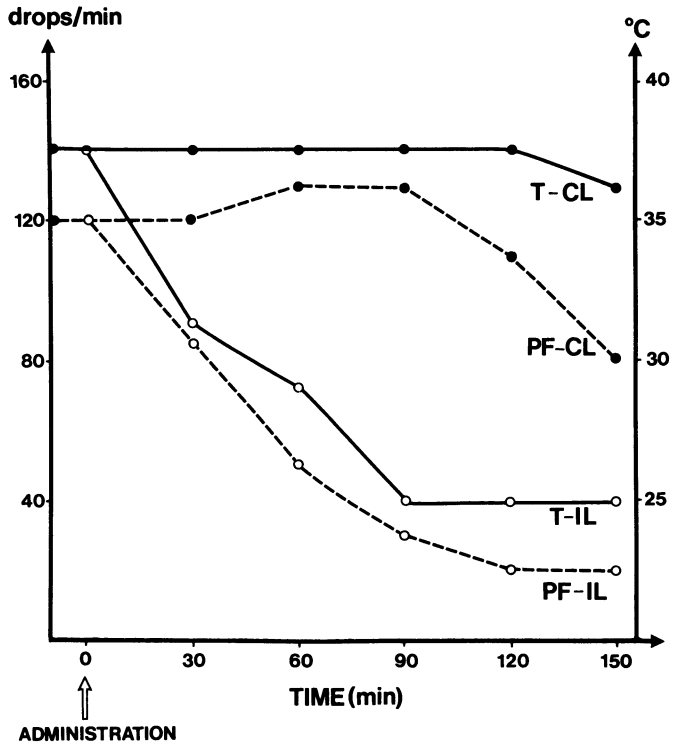

Figure 2. Temperature and perfusate flow in 2 isolated rat liver preparations. T = temperature. PF = perfusate flow. CL = control liver, no blue-green algal extract added. IL = intoxicated liver, 2.0 ml (50 mg/ml) Oscillatoria agardhii-extract from lake Gjersjøen added.

in shape, with a pale cytoplasm, and degenerative changes in the nuclei were also found. Isolated hepatocytes and cytoplasmic fragments were seen in the lumens of the sinusoids and larger blood vessels. No such pathological changes were seen on isolated control livers. Administration of extracts of material from lake Årungen gave the same results as those seen with control preparations.

Effects on isolated heart preparation

Injection of a lethal dose (25—50 mg) of extract of material from lake Gjersjøen constantly produced a significant negative chronotropic effect in rat hearts, lasting up to 10 min after injec-

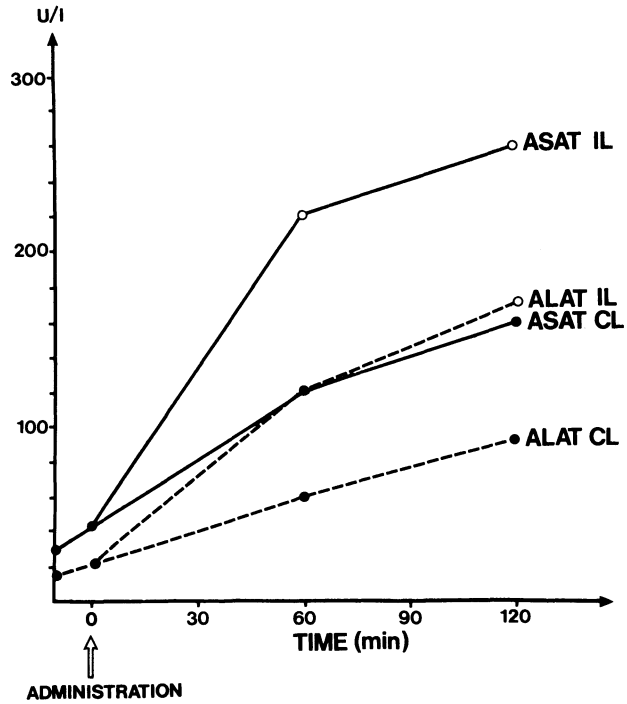

Figure 3. Perfusate plasma values of ASAT and ALAT in 2 isolated rat liver preparations. CL = control liver, no blue-green algal extract added. IL = intoxicated liver, 2.0 ml (50 mg/ml) Oscillatoria agardhii-extract from lake Gjersjøen added.

tion. There was also a shorter period of moderate negative inotropic effect. With extracts from the lake Årungen material, weak with the finding of intact erythrocytes in in vivo experiments.

Effects on isolated ileum preparation

Extracts of material from lake Gjersjøen or from lake Årungen did not influence the contraction properties of the preparation (doses of 50—200 mg).

Effects on isolated phrenic nerve diaphragm preparation

Neither extracts of algae originating from lake Gjersjøen nor from lake Årungen produced any effect on the preparation (doses of 50—200 mg).

DISCUSSION

Our results indicate that the Oscillatoria agardhii-toxins do not possess any hemagglutinative or hemolytical properties. Several Microcystis aeruginosa-toxins have been tested in the same way, with somewhat contrasting conclusions (*Carmichael & Bent* 1981, *Grabow et al.* 1982, *Runnegar & Falconer* 1982). As the latter authors state, the negative findings are in accordance with the finding of intact erythrocytes in in vivo experiments.

The immediate rise in blood pressure registered after adminstration of the toxin is assumed to be caused by an increase in peripheral vasculatory resistance. The contractive force of the heart itself, as seen in the isolated organ study, is in fact reduced, and the injection volume alone does not cause any increase in blood pressure. In a similar study with Microcystis aeruginosa-toxin, the result was an initial fall in blood pressure, thought to be a result of the direct effect on the heart. The subsequent steady decrease was, as here, considered to be secondary to the decrease in circulatory blood volume (*Østensvik et al.* 1981).

The results obtained from experiments with isolated organ preparations clearly show that the liver is the main target of the Oscillatoria agardhii-toxins. The rise in ASAT and ALAT values, fall in glucose concentration and decrease in functional properties of the liver all point in the same direction. AP, γ-GT and bile acid values did not seem to respond to the rapid intoxication. Similar effects were found in an isolated liver preparation study with Microcystis aeruginosa-toxins (*Østensvik et al.* 1981). The histological examinations also revealed serious damage to this organ, the type of liver damages being the same as seen in intoxicated intact animals (*Berg & Søli* 1985). This indicates that the toxins act as direct hepatotoxic agents.

It is concluded from the results of the isolated heart preparation experiment that the Oscillatoria agardhii-toxins have a transient negative effect on contraction force and frequency. The same was found with Microcystis aeruginosa-extract (*Østensvik et al.* 1981). However, slight response was also found with extracts of the non-toxic material from lake Årungen. This effect may differ from the hepatoxic effect, or the lake Årungen material may be slightly toxic, but not enough to be detected by the bioassay methods used.

The Oscillatoria agardhii-toxins did not influence isolated

nerve-striated muscle or smooth muscle preparations. The hind quarter paralysis and periods of convulsions seen in intoxicated intact animals (*Berg & Søli* 1985) must be caused by other, less direct mechanisms, probably as a result of the development of shock. The same findings were done in a study on Microcystis aeruginosa-toxins (*Østensvik et al.* 1981).

Results from the studies performed on isolated laboratory animal preparations were in accordance with the findings on intact animals (*Berg & Søli* 1985). The Oscillatoria agardhii-toxins produced effects which were essensially the same as those produced by the Microcystis aeruginosa-toxins. The main mechanism of action seems to be that of a direct hepatotoxin, with some additional effects on the heart and blood pressure.

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SAMMENDRAG

Virkninger av Oscillatoria agardhii-toksin på blodtrykk og isolerte organpreparater.

Toksiske ekstrakter av blågrønnalgen Oscillatoria agardhii ble undersøkt m. h. p. effekt på ulike isolerte laboratoriedyrpreparater. Oscillatoriatoksinet viste ikke hemagglutinerende eller hemolyserende egenskaper. En umiddelbar blodtrykksstigning ble sett etter intravenøs injeksjon på rotter. På isolerte organer fant en hovedsaklig effekt på leveren. Tilsetning av toksisk ekstrakt til leverperfusjonsløsningen ga fysiologiske, biokjemiske og patologiske forandringer som viste alvorlig skade på organet. På isolert hjerte fant en en negativ kronotrop og inotrop effekt. Toksinene virket ikke inn på isolert glatt muskel eller nerve-tverrstripet muskelpreparater.

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