Effect of glomerulopressin on the lymphatic hearts of the toad, probably mediated by prostaglandins

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Summary. Glomerulopressin acts on the lymphatic hearts of toads: increasing the passage of T-1824 from the abdominal lymphatic sac to the veins in normal animals, but not in toads treated with indomethacin.

Glomerulopressin has been found so far in the ultrafiltrate of plasma circulated through toad liver[1], in the plasma ultrafiltrate of blood obtained from the hepatic vein of rabbits following plasma volume expansion[2], in depancreatized dogs[3] and in normal and depancreatized dogs after infusion of glucagon into the portal vein[4].

Ultrafiltrate hydrolyzed with β-glucuronidase produced complete loss of glomerulopressin activity. However, β-glucuronidase inhibited by boiled saccharate, a well known specific blocker of this enzyme[5], failed to alter glomerulopressin activity[3]. These findings suggested that glomerulopressin is a glucuronide.

The lymphatic hearts of amphibians have a fundamental role as propellers of the lymph from the lymphatic spaces towards the venous system. Their destruction produces an accumulation of lymph in the lymphatic spaces which leads to the death of the animals[6].

The present experiments were performed to study the effect of glomerulopressin on the activity of the toads' lymphatic hearts, and to ascertain whether this effect was mediated by prostaglandin synthesis, as in other systems.

Materials and methods. Male toads (*Bufo arenarum* Hensel) weighing between 100 and 180 g were used. Anesthesia was induced using 20% urethane. Catheters were placed in the aorta and in the abdominal lymph sac.

A bolus injection of 10 ml per 100 g b.wt of T-1824 (Warner-Chilcott) at a concentration of 0.5 mg/ml of T-1824 with 0.8 mg/ml egg albumin (Merck) was injected into the abdominal lymphatic space and 1 ml of concentrated active or inactivated glomerulopressin, or saline, was simultaneously injected through the aorta. At 10, 20, 40, and 80 min of the injections, 1 ml blood samples were obtained from the aorta. Plasma was diluted with saline (0.1 ml plasma was diluted to 3 ml), and read at 620 nm.

Treatments. A group of toads was given 10 mg/kg b.wt of indomethacin (Merck, Sharp and Dohme) 24, 18 and 1 h before the assay. Another group of toads was injected with 13.65 mg/kg b.wt of tranylcypromine (Smith Kline and French Labs) 1 h before the assay. In another group of toads the 4 lymphatic hearts were destroyed by cauterization following the method of Foglia[7].

Glomerulopressin was obtained as previously described[8], and inactivated with β-glucuronidase (Ketodase Warner-Chilcott).

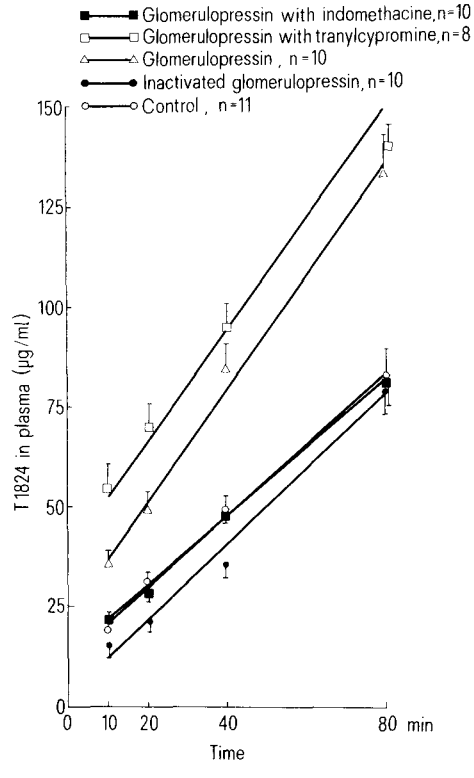
Results. When 1 ml saline was injected through the aorta simultaneously with the injection of T-1824 into the abdominal lymphatic sac, in the 10-min reading there was a significant amount of T-1824 in the aortic plasma. There was a linear increase of T-1824 during the 80 min of the experiment (figure). The injection of glomerulopressin produced a significant increase in the plasma concentration of T-1824 in all the readings. Inactivated glomerulopressin was injected to confirm that the effect on the lymphatic hearts was due to glomerulopressin and not to another substance contained in the ultrafiltrate. The results obtained with inactivated glomerulopressin were not statistically different from those seen in the animals injected with saline (figure). When lymphatic hearts were destroyed by cauterization, the passage of T-1824 was completely inhibited (table).

The treatment with indomethacin inhibited the effect of glomerulopressin, there was no statistical difference with the values obtained in animals injected with saline. In tranylcypromine-treated animals glomerulopressin had the same effect as in nontreated animals (figure).

Concentration of T-1824 in the plasma of normal toads, and in the plasma of toads in which the lymph hearts have been destroyed by cauterization

Minutes	Normal toads (N = 11) µg/ml	p-value	Toads with destroyed lymp hearts (N = 5) µg/ml	p-value
10	19± 4.1	0.001	0±0	-
20	31± 6.2	0.001	2±1.4	NS
40	49± 8.5	0.001	4±2.3	NS
80	82±15.0	0.001	7±3.1	NS

Values are expressed as means ± SE. N, number of toads in each group; p, indicates significance of difference with zero in each group.



Regression lines between the aortic plasma concentration of T-1824 and time. Each point represents the mean of the group, vertical bars represent the SE of the mean, N = number of animals in each group. There is no statistical difference between the control group and the group of animals injected with inactivated glomerulopressin or the group of animals treated with indomethacin and injected with glomerulopressin. The concentration of T-1824 in the animals injected with glomerulopressin was significantly higher that in the control animal ($p < 0.05$). The response to glomerulopressin in the group treated with tranylcypromine was not significantly different to that of nontreated animals.

Discussion. It was observed that glomerulopressin increased the rate of passage of T-1824. That this effect was due to glomerulopressin itself was suggested by the fact that after inactivation with β-glucuronidase the rate of passage was similar to that in the control animals (figure).

That the only route for the passage of T-1824 was through the lymphatic hearts was suggested by the observation that when they were destroyed the dye did not appear in the blood (table).

Recent reports have documented that tranylcypromine reduced the activity of prostacycline synthetase[9].

Tranylcypromine did not alter the effect of glomerulopressin, therefore it can be deduced that glomerulopressin did not stimulate prostacycline synthesis.

Indomethacin is an inhibitor of prostaglandin synthesis[10]. In other studies it has been shown that glomerulopressin acts through the synthesis of prostaglandins, for example in isolated strips of stomach fundus, duodenum and bladder[11], in ovarian blood flow[12] and mesenteric blood flow (in preparation).

In this study it was observed that the treatment with indomethacin completely inhibited the effect of glomerulopressin (figure) suggesting that the effect of glomerulopressin on the toad's lymphatic heart was also due to a stimulation of prostaglandin synthesis.

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Expansibility of erythrocytes during the course of hypotonic hemolysis

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Summary. A quick method for measuring the expansibility of erythrocytes during the course of hypotonic hemolysis has been developed, using the MCV continuous analyzer. By this method not only the volume changes during hypotonic hemolysis, but also the critical volume, at which hemolysis of the cells occurs, could be measured in a few minutes. Furthermore, data are presented demonstrating the following; when the MCV or the MCH was larger, the critical volume increased, but the expansion ratio (critical volume/initial volume) was almost constant, about 1.90, for most erythrocytes.

Erythrocytes increase their volume under hypotonic conditions and hemolysis occurs at the critical volume of the cells[1,2]. The main determinants of in vitro hemolysis are the critical volume and the total number of intracellular osmotically active constituents. The critical volume is a useful parameters; it is dependent on quantitative and qualitative factors associated with the membrane lipid and protein[3]. The determination of the critical volume is a good way to analyze the mechanism of hemolysis. Usually, the microhematocrit method is used to measure the volume. But the method is not convenient and not very accurate; the procedure is very time-consuming, and Guest and Wing's correction has to be used to calculate the critical volume[4]. Therefore, we developed a new direct method to measure volume changes (MCV) during hemolysis, and the critical volume, as reported in this paper.

To measure cell volume, the MCV continuous analyzer (TOA Medical Electronics Co., Kobe-Los Angeles-Hamburg), which can measure mean cell volume and record it continuously at time intervals of 1–10 sec, was employed. The principle for measuring cell size is the same as that of the Coulter counter[5]. But the machine has 2 kinds of special circuits; a conductivity-error compensation circuit and a temperature-error compensation circuit. The machine can determine cell-volume accurately (determination range; 1–1000 fl/cell) in hyper- or hypo-tonic media or at low or high temperatures (4–40 °C)[6,7]. We installed a water pump in the machine to dilute the cell suspensions. Before every experiment we checked the compensation functions by using standard resin particles.

2 µl of heparinized blood were suspended in 40 ml of Celluent (TOA Medical Electronics Co., cell diluent for counting blood cell number, 265 mOsM). After checking the stability of the machine for 15 sec, distilled water was added continuously at a flow rate of 0.5 ml/sec into the cell suspension. Changes in mean cell volume were measured at 5 sec intervals and recorded on a chart.

By the above procedure hemolysis curves like that shown in figure 1 could be obtained. On the chart V_0 means the initial volume (MCV; mean corpuscular volume), water ↓ means the starting point of water addition, p indicates the peak point of the curve, and V_1 means the maximal expansion volume. The curve was much the same as Seeman had proposed previously[1]. The maximal expansion volume, V_1, corresponded to the critical point of Seeman, at which hemolysis had occurred. The time from the starting point of water addition (0 sec) to point p (t sec) could be converted into osmolarity (h) of suspending medium by the following formula; h (mOsM) $= 265 + 1.656 \times t$. Mean osmolarity of the hemolysis point in erythrocytes obtained from 239 subjects was 135.8 ± 22.3 mOsM. The value is very close to the 50% hemolysis value obtained by Dacie's method[8].

The degree of expansibility during hypotonic treatment was determined by the height of p (V_1) and time to p (t). The ascending foot of the curve in figure 1 could be expressed