Frequent Administration of Uric Acid Extends Survival of Fasting Analbuminemic Rats under Cold Environment

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Abstract 1. Analbuminemic rats died within 18 h after a rapid decrease of body temperature whereas control Charles River, Wistar, and Sprague Dawley rats survived for 40 h, when the animals were kept at 5°C without food. 2. Five low molecular weight fractions obtained from Sprague Dawley rat sera were administered to analbuminemic rats kept under these conditions. The duration of survival was extended by the administration of two of the fractions. 3. Several characteristics of one of these fractions coincided with those of uric acid, and body temperature of analbuminemic and Sprague Dawley rats increased within 5 min after uric acid administration.

Key words: uric acid, fast, analbumin, cold, body temperature.

Twenty one analbuminemic subjects were reported to live healthy lives without severe illness symptoms (Russi and Weigand, 1983). Furthermore, Nagase analbuminemic rats (NAR) do not differ from normal Sprague Dawley rats (SDR) in terms of body weight, litter size, or other reproductive characteristics (Nagase et al., 1979, 1980; Esumi et al., 1979). The survival and normal functioning of an analbuminemic animal is contrary to the general concept that serum albumin is essential for the maintenance of life. Experimental animals such as rats, are generally bred under optimal temperature and food conditions. On the other hand, in their natural environments, animals are thought to often be subjected to adverse conditions for life maintenance such as cold, heat, or lack of food or water. If analbuminemic rats kept under the above adverse conditions die faster than normal rats, it might mean that death under such conditions is the reason for the non-existence of wild animals defective in the albumin gene. This possibility was tested in the present study.

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MATERIALS AND METHODS

All experiments were conducted using female rats; NAR and SDR from Clea Japan (Tokyo), Charles River rats from Charles River Japan (Atsugi), Wistar rats from Kiwa experimental animals (Wakayama). All animals were fed Oriental Prina chow MF (Tokyo) for 2–3 weeks. For 3 days before the experiments, the rats were fed only at night. Eight-week-old rats were deprived of food or water and transferred at 09:00 to a cold room (5°C) or to a room in which the temperature was maintained at 25°C. Humidity of the room was not controlled. For water drinking experiments, distilled water of corresponding temperature was given. The condition of the animals was checked at 2–6 h intervals.

When animals were fed with a special diet, a mixture of glucose (Katayama, Osaka)-McCollum’s salt mixture (Nakarai, Kyoto)-vitamin mixture (Tanabe, Osaka) = 64 : 13 : 3 (by weight) or a mixture of soy bean oil-McCollum’s salt mixture-vitamin mixture-cellulose powder (Toyo Roshi, Tokyo)-agar (Katayama, Osaka) = 10 : 6 : 1 : 6 : 6 was given.

Body (rectal) temperatures of SDR and NAR were taken at 5–7 h intervals using an electric thermometer (Nihon Kohden, Tokyo).

Approximately 300 ml of pooled sera from 60 SDR (fed ad lib) were dialyzed against 3 l of 0.3% NaCl for 30 h. The outer solution containing molecules smaller than 10,000 daltons was lyophilized and suspended in a small amount of distilled water. The sample was loaded on Sephadex G10 (2.4 x 42 cm) and eluted with 0.085% NaCl (1 tube = 10 ml); because of the small capacity of the column, 1/5 volume of the sample was separated in each chromatography and then the corresponding fractions were combined after 5 chromatographies. Each fraction was concentrated to 1/10 volume by lyophilization and injected intraperitoneally at 2.5 h intervals to NAR kept without food in the cold room.

RESULTS

NAR lived as well as control SDR, Charles River rats, and Wistar rats for over 60 days, when the animals were kept at 5°C with free access to food and water (Fig. 1). On the other hand, when food was withheld, the NAR died prior to three control rats, independent of temperature conditions. Differences in duration of survival were most pronounced when rats were kept in a cold environment without food (NAR died within 18 h). Furthermore animals fasting only with water died sooner than animals with parallel deprivation of food and water; this is in agreement with our observations for other species (unpublished data). Body (rectal) temperature of NAR and SDR under cold conditions decreased gradually from 15 h preceding death (Fig. 1B).

Mixtures of several kinds of nutritional constituents were administered to fasted NAR to examine which substance can extend their survival (Fig. 2). Administration of glucose and lipid extended their survival but the administration
Fig. 1. A: survival of SDR (●), NAR (×), Charles River (△), and Wistar (○) rats kept with or without food or water. Female NAR and normal control rats were fed Oriental Prina chow MF (Tokyo) for 2–3 weeks. For 3 days before the experiments, the rats were fed only at night. Eight-week-old rats were deprived of food or water and transferred at 09:00 to a cold room (5°C) or to a room in which the temperature was maintained at 25°C. Humidity of the room was not controlled. For water drinking experiments, distilled water of corresponding temperature was given. The condition of the animals was checked at 2–6 h intervals. B: body (rectal) temperature of female SDR and NAR kept in the cold room. The temperature of each animal was taken at 5–7 h intervals using an electric thermometer (Nihon Kohden, Tokyo).
of salts or vitamins did not. This suggests the sudden death of fasted NAR is due to the depletion of energy source such as sugar or lipid or due to a defect of energy production which is caused by a lack of some substance like a hormone which activates energy production from an endogenous source. NAR seems to have the same amount of subcutaneous fat as control SDR, because the body weight of NAR is almost the same as SDR (NAGASE et al., 1980). Therefore, it is expected that administering to NAR such substance present in the blood of the control rats would extend their survival.

Small molecular fractions from mixed SDR sera were obtained by dialysis and were further fractionated by Sephadex G10 (Fig. 3A). Each fraction was administered to NAR kept without food at 5°C with free access to water. Figure 3B showed that the NAR, administered fraction 3 or 5, survived longer than those administered 0.9% NaCl or other fractions. Thus, fractions 3 and 5 were thought to contain humoral factors which are present in SDR but not in NAR blood, and which may play an active role in the body's heat producing system.

Fraction 3, which is a mixture of many substances, was not analyzed in the present study. Fraction 5 and 20 kinds of amino acids were chromatographed on silica gel plate. A small spot was detected with Ninhydrin of which Rf value coincided with that of tryptophan. Another large spot was visible under day light but this was not detectable with Ninhydrin. Fraction 5 was analyzed for its ultraviolet absorbance characteristics at acidic, neutral, and alkaline pH's (Fig. 4).
Fig. 3. A: separation of rat serum small molecular fractions by Sephadex G10 with 0.085% NaCl. Approximately 300 ml of pooled sera from 60 SDR (fed ad lib) were dialyzed against 3/ of 0.3% NaCl for 30 h. The outer solution containing molecules smaller than 10,000 daltons was lyophilized and suspended in a small amount of distilled water. One fifth volume of samples were loaded on Sephadex G10 (2.4 x 42 cm) and eluted with 0.085% NaCl (1 tube=10 ml); following this, each fraction from 5 chromatographies was combined. Glucose ( ) in fraction 2 and NaCl ( ) in fraction 3 obtained from the initial serum sample were eliminated as much as possible by rechromatographies under the same conditions. B: effects of administrations of the fractions on survival of NAR kept without food in a cold room (5°C). Each fraction shown in A was concentrated to 1/10 volume. Concentrated fractions, NaCl, and glucose solutions (0.8 ml) were administered i.p. at 2.5 h intervals to female NAR at 8 weeks of age.
The characteristics of this fraction did not agree with those of tryptophan and were quite similar to those of uric acid. Absorption spectrum of a rechromatographed sample of fraction 5 agreed with that of uric acid. The mobilities of uric acid and tryptophan were determined using the same column used in fractionation of serum samples (Sephadex G10). The mobility of uric acid agreed with that of fraction 5 and tryptophan migrated just after uric acid. Therefore, commercially available uric acid and its degradative products, allantoin, allantoic acid, and urea, were

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Fig. 6. Change of body (rectal) temperature in NAR and in SDR after injection of uric acid. One ml suspension of 2% uric acid in 0.9% NaCl, or 0.9% NaCl, was injected subcutaneously into the back of each animal at 12:50–13:30 and rectal temperatures were taken at intervals. Animals were kept at 23, 17, or 15°C.
administered to NAR kept at 5°C without food. Survival of the NAR administered uric acid was prolonged compared to NAR administered other substances whose duration of survival was similar to that of controls (0.9% NaCl) (Fig. 5).

Injection of uric acid suspension into NAR increased the body (rectal) temperature about 0.6°C while the injection of 1 ml 0.9% NaCl increased body temperature only 0.2–0.3°C; it decreased 0.2–0.3°C when room temperature was at 17°C (Fig. 6). Effect of uric acid administration on the body temperature was more pronounced in SDR: it increased more than 1°C after uric acid injection.

**DISCUSSION**

All rats lived for over 60 days at 5°C when food and water were given freely. Moreover, NAR lived for over 10 days with free access to water and a mixed powder of glucose, salts, and vitamins; they lived for 3 days with lipid, salts, and vitamins, when they were kept at 5°C (Fig. 2). Thus, the reason for the death of the animals is thought to be the depletion of fuel (energy source), such as sugar or lipid. NAR died within 30 h when 1 ml of 6% glucose was injected intraperitoneally at 3 h intervals (Figs. 3 and 4), while NAR with free taking of glucose survived more than 10 days (Fig. 2). This suggests that a large amount of glucose is needed to survive.

When the animals are starved, blood sugar level decreases rapidly and the energy is mainly produced from lipid: fatty acids, glycerol, and ketones (Martin et al., 1983). Because the lipase system cannot act without plasma albumin, energy production from lipid is thought to be depressed in NAR. When obese hyperglycemic mice (C57BL/6J-ob/ob), which have depressed lipid oxidation capacity (Coleman and Hummel, 1972), were kept without food at 24°C, they survived for only 6 days despite the presence of many lipids in adipose tissue; with administration of 5 mg uric acid every day to these mice under fasting conditions their survival time extended to 30 days.

Data obtained in the present study suggest that uric acid activates a heat or energy (ATP) production system(s), perhaps from lipid. This concept of the role of uric acid is applicable for understanding the characteristics of hyperuricemic or gouty humans. Population studies on the characteristics of hyperuricemic and gouty humans showed that such persons are less susceptible to certain kinds of fatigue and show higher activity in drive, achievement, and leadership (Brooks and Mueller, 1966; Lane et al., 1969; Mueller et al., 1970; Kasl et al., 1970a, b; Katz et al., 1975). All these characteristics of hyperuricemic persons could be understood by the higher activity for energy production. The small differences in serum uric acid levels between hypouricemia (ca. 3 mg/dl) and hyperuricemia (ca. 9 mg/dl) suggest a particular state of uric acid which affects the energy production system in particular organ(s) of hyperuricemic humans.

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REFERENCES


