Congenital deficiency in plasma kallikrein and kininogens in the brown Norway rat

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Summary. The kallikrein-kininogens-kinins system has been investigated in the brown Norway rat. In this breed the stores of kininogens in the plasma are reduced and the plasma kallikrein-like activity appears to be absent both in vivo and in vitro.

Various factors of the kinin system have been shown to be involved in blood coagulation in humans. Normally the contact of plasma with a negatively charged surface, like glass or kaolin, activates the Hageman factor (factor XII)[3-5]. This activation requires the presence of the Fletcher factor (prekallikrein)[6,7], which itself is stimulated by factor XII, and of the Fitzgerald factor (high mol.wt kininogen)[8]. Several of these factors have been shown to be absent in the plasma of patients, and disorders of blood coagulation, fibrinolysis and kinin formation ensued. Similarly, plasmas deficient in 2 types of kininogens have been described in humans[9-12].

The brown Norway rat reacts in an unusual way to kinin forming agents. As will be shown the plasma in this breed is congenitally devoid of prekallikrein and deficient in kininogens; this makes these rats interesting experimental animals for the study of the role of the kinin system[12-14] in various physiological and physiopathological processes.

Material. Brown Norway rats *(Rattus norvegicus)* (BN/Mai Pfd f) weighing about 210 g, were obtained from the Katholieke Universiteit of Leuven (Heverlee, Belgium). Wistar rats (W) bred in our laboratory were used as controls.

Results. 1. The drop in arterial blood pressure induced by administration of bradykinin is slightly less marked in BN than in W. The 2 breeds have about the same initial blood pressure 94.4 ± 7.10 mm Hg for W ($n=7$) and 103.9 ± 4.40 mm Hg in BN ($n=10$). Injection of 12 µg/kg bradykinin i.v. lowers the blood pressure by $37.20 \pm 2.35\%$ in W and $21.14 \pm 2.26\%$ in BN ($t=4.81$; $p<0.001$).

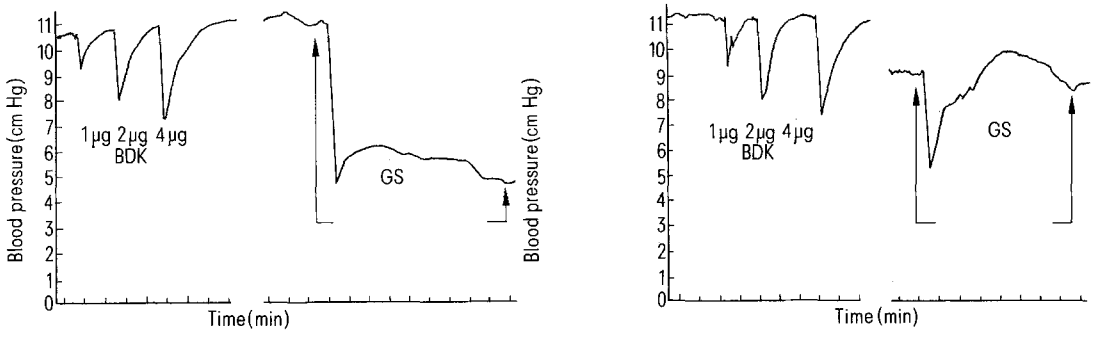
2. The situation is different when kinin forming agents are administered. I.v. injection of 150 µg/kg ellagic acid[16-18] in W causes a fall of more than half the blood pressure level. This response is subject to tachyphylaxis and fades after 3 or 4 successive injections, revealing the progressive consumption of the HMW kininogen[17-19]. On the other hand, in BN ellagic acid is devoid of activity ($n=14$). Rats born from the mating of a BN female and a W male are characterized by a white patch in the fur of the lower abdomen. When tested at the age of 2 months these hybrids reacted to ellagic acid like normal W rats of the same age.

3. In W rats slow i.v. infusion of kallikreins extracted from salivary glands[20,21], originating from either W or BN, provokes an immediate fall in blood pressure which remains at a low level for a prolonged period. The same procedure in BN lowers the blood pressure by 40-50 mm Hg but this drop is transient and the normal level is recovered in 2-3 min (figure). The planimetric estimation of the hypotension recorded allows us to calculate that the glandular kallikreins release an amount of vasodilator substances equivalent to 40-100 µg bradykinin in W and only 4-8 µg in BN ($n=5$).

4. In order to explain the low activity of the kinin forming agents in BN rats in vivo, the total plasma content in kininogens has been estimated in both strains by the method of Diniz and Carvalho[22]. The kininogens amount to 3.82 ± 0.22 µg bradykinin equivalents in W ($n=8$) and 1.13 ± 0.30 µg in BN ($n=6$) ($p<0.001$).

5. The plasma of W, incubated at 37 °C during 10-30 min with ellagic acid (20-50 µg/ml) or carrageenan iota[23] (10 µg/ml or more) in the presence of a kininase-inhibitor phenanthroline ($4 \cdot 10^{-3}$ M)[24], proved to contain kinins. After this treatment, the W plasma contracts the guinea-pig ileum and relaxes the rat duodenum superfused with a Tyrode solution containing atropine and promethazine ($1 \cdot 10^{-6}$)[25]. The kinins formed amount to 1.2-1.8 µg/ml. By contrast the BN plasma incubated under the same conditions was devoid of activity and thus no kinins were formed ($n=8$). Consequently ellagic acid is inactive in BN rats in vitro as well as in vivo.

6. If an aliquot (0.5 ml) of a solution made of 10 µg carrageenan in 1 ml W plasma, diluted with NaCl 0.9 g% to a final volume of 2 ml, is added after 1 min of incubation to 1 ml W plasma containing phenanthroline, 1-1.8 µg/ml kinins are produced. The addition of the same amount of carrageenan (2.5 µg) directly to W plasma is ineffective. Thus a kinin-forming agent is present in W plasma which is activated by carrageenan to produce kinins[26]. Under identical conditions addition of the same aliquot of kinin-forming agent from W plasma to 1 ml BN plasma produces at most 0.2 µg/ml kinins. This small quantity could be attributed to the W plasma contained in the aliquot. There



Blood pressure recording. Left: Wistar rat; right: Brown Norway rat. W rats (225 g) and BN rats (210 g) anaesthetized with pentobarbital (3 mg/100 g). Bradykinin injection (BDK). Slow infusion (1 ml/4 min) of a salivary gland homogenate (GS) (100 mg/ml) coming from W rats.

is thus little or no substrate for kinin-forming agents in BN plasma. The same procedure, this time using BN plasma as a source of kinin-forming agents, does not produce kinins.

7. Dextran sulfate (mol.wt 500,000) activates the Hageman factor which in turn accelerates the conversion of prekallikrein into kallikrein[27,28]. These processes can be estimated by the method of Amundsen and Svenden[29] which measures the amidolytic potency of the kallikrein formed. In W plasma this activity is 0.449 ± 0.047 units/ml, whereas it is nil in BN plasma.

Discussion. BN plasma is characterized by its low content of kininogens. This has been demonstrated directly, and confirmed by the fact that salivary gland kallikreins possess only a weak hypotensive activity in BN rats. Moreover if kallikrein is activated by carrageenan in W plasma and then transferred to BN plasma, little or no kinin is formed in the latter, indicating that HMW kininogens are practically absent from BN plasma.

BN plasma is also devoid of kallikrein. This conclusion is supported by 3 pieces of evidence: a) ellagic acid has no hypotensive activity in BN rats in vivo; b) ellagic acid and carrageenan iota fail to produce kinins from BN plasma in vitro; c) no amidolytic activity can be demonstrated in BN plasma when prekallikrein is activated by means of dextran sulfate. As ellagic acid, carrageenan and dextran sulfate activate prekallikrein through the activation of the Hageman factor[16,28,29] and as this factor is present in W and BN plasma[30], it seems most probable that the lack of kallikrein in BN plasma is responsible for the inactivity of these 3 compounds.

On the other hand, kallikreins are found in the salivary glands of both BN and W rats. Homogenates of the glands in BN rats exert a hypotensive activity in W rats and cross tachyphylaxis can be demonstrated on the vascular effects of BN and W homogenates.

These results provide a logical explanation for the fact that inflammatory processes caused by 3 different carrageenans are less marked in BN than in W rats[30]. It is thus hoped that the use of the BN strain may throw some light on the role of the kinin system in other physiological or pathological processes.

Conclusion. The plasma of the brown Norway rat is devoid of kallikrein and poor in kininogens, but in the salivary glands of this breed, glandular kallikreins are present.

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Candida utilis as a convenient and safe substitute for the pathogenic yeast *C. albicans* in Daniels' phototoxicity test

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Summary. Candida utilis is a safe and convenient substitute for the pathogenic yeast *C. albicans* in phototoxicity tests. With both organisms 8-methoxypsoralen and α-terthienyl give positive results while photodynamic compounds give negative results.

Since Daniels introduced *Candida albicans* as a test organism for determining whether plant materials or isolated chemicals displayed an antibiotic activity in the presence of long wavelength UV-light[2], the use of this phototoxicity test has grown, especially since the phototoxic psoralens have been applied in medicine[3]. For example, Towers and his coworkers have tested extensively for the presence of phototoxic compounds in several plant families, with emphasis on the Compositae[4–6].

It is appropriate to stress that *C. albicans* is a pathogenic organism which has been implicated in many different types of infections[7]. A superficial candidiasis may be either cutaneous, affecting for example the skin or the nails, or it may be mucosal, affecting the digestive, genital, urinary or