Developmental Susceptibilities of Rabbits to Various Biological Activities of Bacterial Endotoxin

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Abstract. The developmental pharmacology of endotoxin (Escherichia coli UKT-b) in the rabbit was studied. When newborn rabbits were kept at a high ambient temperature at which its basal metabolism was considered minimal, a small but definite febrile response was observed to endotoxin administered intravenously (0.5 µg/kg), subcutaneously (1 µg/kg) or intracisternally (0.1 ng/kg). At room temperature (25°C), such a significant febrile response was not observed. The febrile response increased in magnitude with the advance in age. Susceptibilities to the lethal effect of intravenous or intracisternal endotoxin were high in rabbits from the 1st to 3rd day of life. They decreased over a period from the 7th to 21st day of life. Thereafter, they increased with the advance in age. Neither skin reaction nor shock by endotoxin was observed in rabbits under 21 days of age. These results suggested that the receptor-effector mechanisms to endotoxin might be developed fairly in the CNS, but very poorly in the peripheral organs of rabbits in the early postnatal age.

It has been well documented that newborn animals are highly resistant to some effects of bacterial endotoxin, the susceptibilities to which increases with the advance in age [7, 13, 16, 18]. These findings support a hypothesis that the biological activities of endotoxin may depend on the degree of sensitization to endotoxin from the alimentary tract [5, 11, 12, 14, 16].

In contrast to these findings, recent studies on susceptibilities to endotoxin have shown that newborn guinea pigs are more susceptible to the lethal effect of endotoxin than the adults [15], but that no pyrogenic sensitivity is apparent during the first postnatal week [2]. Aside from a possible species difference, the susceptibilities to endotoxin appear to be dependent upon the degree of maturity in the respective target organ. So far as the authors know, no experiments have yet been made to evaluate the exact relationship between the febrile response and any other response to endotoxin in newborn rabbits.

The purpose of the present study is to compare the developmental changes of the various biological activities of endotoxin, including pyrogenicity, lethality, and activities to produce skin reaction and endotoxin shock in rabbits.

Materials and Methods

Animals: Japanese albino rabbits of both sexes —1 to 0, 1, 3, 7, 14, 21, 28, 35 and 70 days of age and male adult rabbits were used. Minus-one-day-old rabbits were neonates obtained by cesarean sec-
tion one day before delivery (harvested on the 30th day of gestation). They were brought up in the laboratory animal room by the doe mated here. The neonates were nursed by their mother until 25 days of age, when they were weaned and allowed to have rabbit pellets and tap water ad libitum. The animal room was maintained at 25±1°C and a humidity of 50±10%.

Endotoxin: The endotoxin used was a lipopolysaccharide (LPS) extracted from *Escherichia coli* UKT-b by the method of Westphal and Lüderitz [17]. It was dissolved in sterile pyrogen-free saline. The chemical properties and pyrogenic activity of LPS were described in detail in the previous report [9].

Measurement of rectal temperature: The rectal temperature was measured by a copper-constantan thermocouple, which was inserted into the rectum and held in the place with adhesive tape wrapped around the tail. Experiments were conducted under two ambient thermal conditions: at a neutral temperature (Tn) and room temperature (25°C). Since the neutral range of ambient temperature (within which the rate of oxygen consumption was minimal in rabbits) varied with age during the first 10 days of life [4], a lowest possible limit of the neutral range (critical temperature) was chosen for Tn as follows:

<table>
<thead>
<tr>
<th>Age in days</th>
<th>0</th>
<th>1</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tn (°C)</td>
<td>35.5</td>
<td>35.0</td>
<td>34.5</td>
<td>33.0</td>
<td>32.0</td>
<td>29.0</td>
<td>27.0</td>
</tr>
</tbody>
</table>

Each rabbit was conditioned for at least 90 min before the injection of endotoxin to establish a baseline rectal temperature. Temperatures were recorded on a multichannel pen-recorder at 30 sec intervals over a period of 5 hr after the injection of endotoxin.

Measurement of blood pressure: Rabbits were anesthetized with subcutaneous injection of urethane (1 g/kg). Heparin (1,000 unit/kg) was used anticoagulant. Mean systemic arterial pressure was measured by means of a catheter inserted into the cervical artery, connected with a pressure transducer and monitored on a writing recorder. Injection was made via a cannula inserted into the cervical vein.

Measurement of skin permeability: For skin testing, 0.025 ml of an endotoxin solution was injected intradermally with 0.5% Evans' blue (2 ml/kg, intravenous injection (i.v.)). Then the bluish area (cm²) was measured after 5 hr (PF-5). The methods used were described in detail elsewhere [10].

All glasswares and needles were sterilized by dry heating at 250°C for 2 hr to inactivate any contaminating pyrogens.

Results

1. Effect of age on pyrogenicity of endotoxin
2. Effect of ambient temperature on rectal temperature in young rabbits

Experiments were made to investigate the effect of ambient temperature on the rectal temperature of rabbits of various ages during the first postnatal month. The results obtained are shown in Table 1. The value given represents the mean ± S.D. of the respective measurement computed over a 30-minute period beginning after the stabilization of rectal temperature. Generally, a stable level was reached within 90 min after the beginning of exposure. It should be noted that during the first 7 days of age the rectal temperature was significantly lower than the neutral ambient temperature. Under the thermoneutral condition, there was no significant difference in rectal temperature during the first month of life in relation to age or environmental temperature. After 3 days of age a rapid increase was occasioned in body weight.

b. Fever in newborn rabbits

<table>
<thead>
<tr>
<th>Age in days</th>
<th>Body weight (g) at Tr (25°C)</th>
<th>Rectal temperature (°C) at Tn**</th>
</tr>
</thead>
<tbody>
<tr>
<td>—1*</td>
<td>51.7±3.8</td>
<td>38.40±0.44</td>
</tr>
<tr>
<td>0</td>
<td>61.7±11.8</td>
<td>34.63±2.48</td>
</tr>
<tr>
<td>1</td>
<td>56.6±6.9</td>
<td>35.87±1.02</td>
</tr>
<tr>
<td>3</td>
<td>81.3±11.5</td>
<td>35.54±2.09</td>
</tr>
<tr>
<td>7</td>
<td>147.9±35.9</td>
<td>36.39±1.02</td>
</tr>
<tr>
<td>14</td>
<td>221.3±65.5</td>
<td>38.20±0.66</td>
</tr>
<tr>
<td>21</td>
<td>322.6±69.4</td>
<td>38.40±0.66</td>
</tr>
<tr>
<td>28</td>
<td>484.1±110.0</td>
<td>38.49±0.66</td>
</tr>
</tbody>
</table>

* Newborn rabbit obtained by cesarean section one day before delivery.

** Neutral ambient temperature by age. It was determined by referring to [4].

Value is expressed as mean ± S.D.
Peripheral administration of endotoxin: Some temperature responses to the intravenous (0.5 µg/kg) and subcutaneous (1 µg/kg) injections of endotoxin were studied in minus-one-day-old rabbits at a neutral ambient temperature. As shown in Fig. 1, in contrast to the typical biphasic febrile response observable in adult rabbits, a monophasic febrile response was commonly observed. No temperature response was brought forth by the saline-treated rabbits. At room temperature of 25°C (Fig. 2-a), however, no febrile response was induced by the intravenous injection of endotoxin (0.5 µg/kg) in 0-day-old rabbits. The febrile response was weaker in those aged from 3 to 14 days at room temperature than at the neutral ambient temperature. Fever-height was measured as the difference in rectal temperature (ΔTre) between the maximum value attained following the injection of endotoxin and the baseline before the injection. Developmental changes in fever-height induced by the intravenous injection of endotoxin (0.5 µg/kg) are shown in Fig. 2-a. Under thermoneutral conditions, the fever-height was about 1–1.5°C in rabbits under 14 days of age, the fever curve being characterized by a monophasic pattern. It
was about 2–2.5°C in rabbits over 14 days of age, showing a typical biphasic curve.

Intracisternal administration of endotoxin: Intracisternal injection (i.c.) of endotoxin was made to rabbits of 1-day-old and adult ones. The results obtained are presented in Figs. 1 and 2-b. Fig. 1 showed the febrile response of the 1-day-old rabbits after intracisternal injection of endotoxin (0.1 ng/kg) at the neutral ambient temperature. Mean fever-height of these rabbits was 1.12°C. The developmental change of fever-height after the injection of endotoxin (0.1 ng/kg) is illustrated in Fig. 2-b. No significant differences were noted in the fever-height.

2. Effect of age on mortality by endotoxin

Peripheral administration of endotoxin

The lethal effect of endotoxin was determined in rabbits −1 to 70 days of age. Newborn rabbits, during the first postnatal week, were kept in an incubator controlled under thermoneutral conditions for 24 hr after the injection of endotoxin. Then they were returned to their mother. The rabbits used in this experiment were not necessarily littermates, but were rather collected at random from available litters of the same age. Rabbits having received endotoxin were not used again to avoid the development of tolerance. The results obtained are summarized in Table 2-a. Seventy-day-old rabbits died within 24 hr after the injection of endotoxin. In contrast, rabbits −1 to 3 days old died rather late. Rabbits 7 to 28 days old were resistant to endotoxin. In them the susceptibility increased with the advance in age. Rabbits surviving for one week after injection appeared to be completely well, and none of them died thereafter.

Intracisternal administration of endotoxin: To examine the neurotoxic effect of endotoxin, rabbits of various ages were injected with endotoxin (200 μg/kg) into the cisterna magna. The experimental conditions used were the same as those of the peripheral administration. The results obtained are shown in Table 2-b. Deaths were occasioned in rabbits 50 and 70 days old within 24 hr after injection, but in 0-day-old rabbits rather late. The lethal effects of endotoxin induced by peripheral or central administration are summarized in Fig. 3. These results indicated that the neonates and the rabbits 50 to 70 days old were highly susceptible to the lethal effect of endotoxin, and that rabbits 7 to 21 days old were less susceptible.
3. Effect of age on responsiveness of blood vessels to endotoxin

Blood pressure changes after endotoxin administration: The effect of endotoxin on systemic blood pressure was examined in rabbits of various ages. The results obtained are illustrated in Fig. 4. When 5 mg per kg of endotoxin was injected intravenously into 70-day-old rabbits, the mean systemic blood pressure began to fall within 0.5–2.5 min after the injection, reaching a minimum within 5 min, and increased to the control value or thereabout within 30 min. No rabbits under 28 days of age showed such pressure fall. No fall in systemic blood pressure was observed in 3-day-old rabbits after the administration of a large dose of endotoxin (10 mg/kg).

Skin permeability induced by endotoxin: The effect of endotoxin on skin permeability was examined in rabbits of various ages. The results obtained are illustrated in Fig. 4. In no rabbits under 14 days of age, the intradermal injection of endotoxin (10 µg) caused an inflammatory reaction. A large dose of endotoxin (100 µg) induced no reaction either in 3-day-old rabbits.

Discussion

The effect of age on the responses of animals to various doses of endotoxin has been studied from many aspects [2, 11, 13, 15, 16, 18]. Little work, however, has been done on it with newborn rabbits during the first postnatal week.

The studies reported here indicated that the responses of newborn rabbits to endotoxin were similar to those of newborn guinea pigs [2, 15]. Newborn rabbits were highly susceptible to the lethal effect of endotoxin, but showed no febrile response at a room temperature of 25°C.

The febrile response of newborn rabbits
was observed at a high ambient temperature. It is well known that newborn rabbits during the first postnatal week have little fur but a large body surface relative to body weight. So the ambient temperature is one of the important factors to assess the febrile response of endotoxin. Hull [4] demonstrated that a neutral ambient temperature (Tn) at which the basal metabolism of the animal was considered to be minimal was high in newborn rabbits maintaining their body temperature by increasing the rate of basal metabolism at a temperature lower than Tn. It seems to be for these reasons that the febrile response of the newborn rabbits was not observed at a room temperature of 25°C, but was at a high ambient temperature (Tn).

At Tn, the newborn rabbits showed a monophasic febrile response after the intravenous injection of endotoxin, and the adult rabbits a typical biphasic febrile response. It has been postulated that the first peak of endotoxin fever may be due to the direct action of endotoxin on the central nervous system, and the second peak of endotoxin fever to the release of an endogenous pyrogen from granulocytes [1, 8]. Accordingly, judging from the time-course, the febrile response of the newborn rabbits during the first postnatal week seems to correspond to the first peak in the case of adult rabbits. So it is assumed that the receptor-effector mechanisms to endotoxin may exist in the central nervous system, but not in the peripheral systems of the newborn rabbits, and that the susceptibility may develop with the advance in age. This assumption is supported by the fact that the newborn rabbit responds with fever to the central administration of a minor dose of endotoxin.

As was reported by previous researchers [16], it was confirmed that young rabbits 28 days old were tolerant to the lethal effect of endotoxin. This study indicated, however, that rabbits in the early stage of postnatal life were highly susceptible to the lethal effect of endotoxin. This high susceptibility was assumed to be due to the central effect of endotoxin. This assumption was supported by the fact that the central administration of a minor dose of endotoxin produced deaths among rabbits 0 to 3 days old. It is well understood that adult rabbits develop hypotension after the intravenous administration of endotoxin and show an inflammatory response to the intradermal administration of endotoxin [3, 6]. Newborn rabbits, however, showed no response in the usual manner, but inflammatory and shock responses occasionally after the administration of endotoxin. It was previously suggested that the inflammatory response induced by endotoxin might have been mediated by permeability factor(s) released from granulocytes [19]. Hence it is possible to assume that no receptor-effector mechanisms to endotoxin may be noted in the peripheral systems of the newborn rabbit.

From these results, it is reasonable to conclude that the receptor-effector mechanisms to endotoxin may exist in the central nervous system, but not in the peripheral systems of the newborn rabbit, which may become susceptible to endotoxin with the advance in age.

References


要 約
家兎の細菌内毒素に対する各種反応性の発育に伴う変化について: 西尾 晃（鹿児島大学農学部家畜薬理学教室）、加納崎三郎（国立衛生試験所大阪支所薬理微生物部）——家兎の細菌内毒素に対する各種反応性の発育を日齢を追って検討した。妊娠30日目の家兎より帝王切開により得た新生兎家兎でも内毒素の静注（0.5 μg/kg）または皮下注（1 μg/kg）により発熱反応を示した。自然分娩して一日後の新生兎家兎（1日齢）に内毒素を大腸内投与（0.1 ng/kg）しても同程度の発熱反応がみられた。上述の発熱反応は基礎代謝が最低となる環境温度では認められなかった。家兎の大腸内投与による死亡率は、0日齢の家兎で70%，7日齢で21%，21日齢で21%死亡，その後日齢の進むに伴い高くなった。静注時もほぼ類似の成績であった。内毒素の静注による内毒素ショックおよび皮内注による皮膚血管通過性亢進反応は、共に21日齢までの家兎ではほとんど認められなかった。これらの成績は、内毒素に対する receptor-effector 機構は既に新生兎家兎の中枢神経系に存在するが、末梢での receptor-effector 機構の発達とは、日齢を要することを示している。