Hypoalgesia in Genetically Hypertensive Rats (SHR) is Absent in Rats with Experimental Hypertension

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SUMMARY In this study a possible relationship between regulatory mechanisms involved in pain and blood pressure control has been investigated in the rat. Spontaneously hypertensive rats (SHR), their normotensive Wistar-Kyoto (WKY) controls, and two experimental models of hypertension together with their appropriate sham-operated controls were tested for their responsiveness to pain. Two methods for measurement of nociceptive responsiveness (hot plate and electric footshock threshold) were used. A diminished responsiveness was observed in both young (still normotensive) and adult (hypertensive) SHR. Pretreatment with naloxone reduced hot plate response times of adult SHR to the level of WKY, indicating opioid receptor involvement. Despite severe hypertension in rats with a renal artery clip and in DOCA-salt treated rats, no reduction of pain sensitivity as compared to sham-operated controls was evident in the hypertensive rats as assessed by both methods. It is concluded, that the higher pain threshold in SHR is probably determined by genetic factors rather than hypertension. (Hypertension 5: 185-190, 1983)

KEY WORDS • nociceptive responsiveness • experimental hypertension

Genetically hypertensive rats appear to be less responsive to noxious stimuli than their normotensive controls. A similar finding has been reported in rats with renal hypertension and in humans with essential hypertension. Therefore a relationship between the central regulatory mechanisms involved in pain sensitivity and blood pressure may exist.

Other data also indicate a possible relationship between these regulatory mechanisms. The hypotensive drug clonidine appears to cause analgesia in rats and a decrease in blood pressure in spontaneously hypertensive rats (SHR), which can be prevented by the opiate antagonist naloxone. Furthermore, clonidine alleviates opiate withdrawal symptoms in morphine-treated animals; in this respect it is also important that opioid peptides are involved in both pain perception and analgesia and central cardiovascular control.

We have studied the response to thermal and threshold electric stimuli of SHR, Wistar-Kyoto (WKY) controls, renal (two-kidney, one clip Goldblatt), and DOCA-salt hypertensive Wistar rats, together with the appropriate sham-operated control animals. These responses were assessed repeatedly during the development of hypertension and on one occasion only in animals with developed hypertension.

Methods and Materials

Male SHR, WKY rats, and regular normotensive Wistar (Wu/Cpb) rats were obtained from Central Breeding Laboratories TNO (Zeist, the Netherlands).

Production of Hypertension
Renal Hypertension

Under ether anesthesia, renal hypertension was induced by the application of a solid silver clip (0.20 mm, i.d.) to the left renal artery of rats weighing 120 to 130 g. Control animals were subjected to the same operative procedure, but no clip was applied.

DOCA-Salt Hypertension

DOCA-salt hypertension was produced by the subcutaneous implantation of deoxycorticosterone (two pellets containing 20 mg each) under pentobarbitone.
(60 mg/kg, i.p.) anesthesia in rats weighing 120–130 g. Physiological saline solution (0.9% NaCl) was given as drinking water. Sham-operated controls were subjected to the same operative procedure but no DOCA-pellets were implanted. These animals were supplied with normal tap water for drinking purposes.

Blood Pressure Measurements

For chronic measurements, systolic blood pressure was assessed in trained conscious rats by the indirect method of tail sphygmography. In some experiments, blood pressure was measured in untrained rats in the same way under light ether anesthesia.

Assessment of Pain Sensitivity

Responsiveness to noxious stimuli was assessed by both a hot-plate method and an electric foot-shock method.

Hot Plate Method

Criteria for reaction to the hot plate method, which is a modification of the technique described by Eddy and Leimbach (54.0° ± 0.1° C), were paw licking and intensive jerking with lifting of or jumping on the hind legs. In adult SHR and WKY, hot-plate response latency was also tested 15 minutes after administration of naloxone HCl (1 mg/kg subcutaneously, in physiological saline solution).

Electric Foot Shock

Response behavior to electric footshock was studied in a perspex box with a grid floor, as described previously. After 1 minute of adaptation, rats were subjected to 24 shocks of various intensities. Each shock lasted 1 second, the interval between shocks being 15 seconds. The short-circuit values of the subsequent intensities were 0.101, 0.027, 0.065, 0.137, 0.034, 0.158, 0.020, 0.182, 0.079, 0.124, 0.014, 0.046, 0.182, 0.079, 0.020, 0.046, 0.137, 0.065, 0.034, 0.046, 0.158, 0.027, 0.124 and 0.101 mA. The order of these shocks was identical for each animal. The response of the animals during the first second after each shock was recorded. The following criteria were used: no response (0); flinch response, a slight movement of one of the legs or the head (F); jerk, run, vocalized, and jump response (R). The jerk response was a quick and powerful movement of the legs, head, or whole body. The run response was a fast displacement forward for more than the rat's own body length. A jump response was recorded when the rat jumped with all four legs from the grid at the same time. A vocalized response was a scream or a squeak.

Spontaneously Hypertensive Rats and Wistar-Kyoto Controls

With the hot plate method, pain sensitivity was assessed in young (3½ week) and adult (12 week) SHR and WKY without prior exposure to this procedure. The young animals were subsequently tested on the hot plate at regular intervals. In these animals the electric footshock experiment was performed at the age of 12 weeks. Two weeks after the initial hot plate experiment, the adult animals were tested again on the hot plate after administration of the opiate antagonist naloxone (1 mg/kg, s.c., 15 minutes before testing).

Renal Hypertensive Rats

Experiment 1. After application of the renal artery clip or the sham operation, the animals were tested on the hot plate at weekly intervals. In the same animals, the electric footshock experiment was performed 4 weeks after the operation.

Experiment 2. Before application of the renal artery clip or the sham operation, the rats were tested on the hot plate on one occasion and after surgery at regular intervals. Three weeks after application the renal artery clip was removed under ether anesthesia. On the first and third day after this second operation, hot plate latencies were also determined.

Experiment 3. Pain sensitivity was assessed in renal hypertensive rats and controls using the hot plate and electric footshock method, each on one occasion only, after hypertension had developed (i.e., 19 and 21 days respectively after application of the renal artery clip).

DOCA-Salt Hypertensive Rats

Experiment 1. At 5 to 8 weeks after operation, responsiveness to thermal noxious stimuli was assessed in the DOCA-salt treated rats and controls at weekly intervals. The electric footshock test was carried out at 7 weeks after implantation or sham operation.

Experiment 2. In DOCA-salt hypertensive rats and appropriate controls, hot plate response latencies and response behavior to electric footshock were studied using both methods on one occasion only after development of hypertension (i.e., 34 and 36 days respectively after implantation of the DOCA-pellets).

Statistical Analysis. Results are expressed as means ± SEM. For statistical analysis Student's t test (two-tailed, unpaired) was used. Where appropriate, comparisons were performed by a two-way analysis of variance with application of Student's t test. Data were considered to be significantly different at the 5% level.

Results

Spontaneously Hypertensive Rats and Wistar-Kyoto Controls

The 3½-week-old SHR showed a significantly longer response latency in the first hot plate test than the age-matched WKY controls (fig. 1). This difference was also observed in repeated tests. The electric footshock test carried out at the age of 12 weeks elicited a significantly greater number of "no responses" and a smaller number of flinch-responses in the SHR as compared to the WKY. The number of other responses were not different. Blood pressure values obtained from the age of 6 weeks onward were significantly higher in the SHR than in the WKY. No difference in
blood pressure was observed at the age of 4 weeks in an additional group of SHR and WKY, values being 108 ± 2 and 109 ± 2 mm Hg respectively. Testing of these rats for pain sensitivity on the hot plate confirmed the initial results.

The adult SHR, when tested for pain sensitivity on the hot plate without prior exposure to this procedure, also showed increased latency times when compared to WKY (table 1). This difference was abolished by pretreatment with naloxone, latency times being 8.3 ± 0.9 seconds and 7.6 ± 0.8 seconds in SHR and WKY respectively.

**Rats with Renal Hypertension**

**Experiment 1**

At weekly intervals after clip application or sham operation, animals were tested on the hot plate. No differences in hot plate response latency times were observed. The response to electric footshock did not differ between the two groups in the fourth week after operation. At the end of this week, systolic blood pressure was measured under light ether anesthesia (tables 1 and 2). Blood pressure in the clipped rats was significantly higher than in the sham-operated controls.

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**TABLE 1.** Hot Plate Latency Time and Systolic Blood Pressure in Adult SHR, Normotensive WKY Control Animals, Renal and DOCA-Salt Hypertensive Rats, and Sham-Operated Control Animals

<table>
<thead>
<tr>
<th>Rats</th>
<th>No.</th>
<th>Hot-plate latency time (sec)</th>
<th>Systolic blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR*</td>
<td>9</td>
<td>15.4 ± 0.9†</td>
<td>214 ± 3†</td>
</tr>
<tr>
<td>WKY</td>
<td>10</td>
<td>7.6 ± 0.5</td>
<td>135 ± 2</td>
</tr>
<tr>
<td>Renal hypertensive§</td>
<td>5</td>
<td>7.8 ± 0.6</td>
<td>198 ± 8†</td>
</tr>
<tr>
<td>Sham-operated</td>
<td>7</td>
<td>7.4 ± 0.5</td>
<td>128 ± 2</td>
</tr>
<tr>
<td>DOCA-salt hypertensive§</td>
<td>16</td>
<td>9.4 ± 0.5</td>
<td>212 ± 5†</td>
</tr>
<tr>
<td>Sham-operated</td>
<td>12</td>
<td>9.1 ± 0.6</td>
<td>131 ± 3</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SEM.
- *Response latencies presented arose from the first test for pain sensitivity on the hot plate.
- †p < 0.01.
- §As indicated under Methods and Materials these animals have been tested on the hot plate several times during the development of hypertension.
- ‡After determining hot plate latencies, blood pressure was measured under light ether anesthesia 4 weeks after application of the renal artery clip.
- ||After determining hot plate latencies, blood pressure was measured under light ether anesthesia 6 weeks after implantation of DOCA-pellets.
Table 2. Responses to the Electric Footshock Test of SHR, Normotensive WKY Rats, Renal and DOCA-Salt Hypertensive Rats, and Sham-Operated Controls

<table>
<thead>
<tr>
<th>Rats</th>
<th>No.</th>
<th>0</th>
<th>F</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR*</td>
<td>11</td>
<td>9.3 ± 0.6</td>
<td>1.7 ± 0.4</td>
<td>13.0 ± 0.4</td>
</tr>
<tr>
<td>WKY</td>
<td>9</td>
<td>4.6 ± 0.3</td>
<td>5.3 ± 0.4</td>
<td>14.1 ± 0.5</td>
</tr>
<tr>
<td>Renal hypertensive‡</td>
<td>5</td>
<td>5.4 ± 0.7</td>
<td>5.4 ± 0.9</td>
<td>13.2 ± 1.2</td>
</tr>
<tr>
<td>Sham operated</td>
<td>7</td>
<td>6.8 ± 0.6</td>
<td>3.6 ± 0.3</td>
<td>13.7 ± 0.7</td>
</tr>
<tr>
<td>DOCA-salt hypertensive§</td>
<td>12</td>
<td>2.7 ± 0.7</td>
<td>5.2 ± 0.4</td>
<td>16.2 ± 0.7</td>
</tr>
<tr>
<td>Sham operated</td>
<td>12</td>
<td>4.1 ± 0.4</td>
<td>4.4 ± 0.4</td>
<td>15.4 ± 0.4</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SEM.

*12-week-old animals (systolic blood pressures 218 ± 2 vs 143 ± 2 mm Hg).
†p < 0.01.
‡4 weeks after application of renal artery clip (systolic blood pressures 196 ± 10 vs 129 ± 2 mm Hg).
§7 weeks after DOCA-implantation (systolic blood pressure 204 ± 5 vs 131 ± 2 mm Hg).
||Presented is the number of observed responses of the indicated category, total number of possible responses being 24 (for details see Methods and Materials). 0 = no response. F = flinch response. R = other response.

Experiment 2

In this experiment the animals were trained for blood pressure measurement, and their response to thermal noxious stimulation was determined before application of the renal artery clip or sham-operation. Twenty-two days after operation, the renal artery clip was removed under light ether anesthesia. Pain sensitivity, as assessed on the hot plate at regular intervals, did not reveal a difference between the hypertensive groups and the sham-operated animals (fig. 2).

Experiment 3

Seventeen days after surgery, systolic blood pressure of the rats was measured under light ether anesthesia, the clipped animals exhibiting a significant elevation of blood pressure as compared to the sham-operated controls (table 3). Hot plate latency times were determined on the 19th postoperative day, and 2 days later the electric footshock test was performed. Both methods did not reveal any significant difference in pain sensitivity between the hypertensive and normotensive groups of animals. Results are summarized in table 3. After these experiments, blood pressure was measured again, results being similar to those obtained before pain sensitivity testing.

![Figure 2](http://hyper.ahajournals.org/)
Table 3. Hot Plate Response Latency Times, Electric Footshock Responses, and Systolic Blood Pressure Values in Renal and DOCA-Salt Hypertensive Rats and Appropriate Controls without Prior Exposure to Pain Sensitivity Tests

<table>
<thead>
<tr>
<th>Rats</th>
<th>No.</th>
<th>Systolic blood pressure (mm Hg)</th>
<th>Hot plate latency time (sec)</th>
<th>Responses to footshock</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal hypertensive</td>
<td>15</td>
<td>201 ± 5</td>
<td>8.6 ± 0.4</td>
<td>6.1 ± 0.5</td>
</tr>
<tr>
<td>Sham operated</td>
<td>9</td>
<td>125 ± 4</td>
<td>8.8 ± 0.6</td>
<td>6.0 ± 0.5</td>
</tr>
<tr>
<td>DOCA-salt hypertensive</td>
<td>7</td>
<td>191 ± 4</td>
<td>9.1 ± 0.7</td>
<td>6.0 ± 0.5</td>
</tr>
<tr>
<td>Sham operated</td>
<td>7</td>
<td>128 ± 5</td>
<td>9.2 ± 0.8</td>
<td>5.3 ± 0.6</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SEM.
Systolic blood pressure was measured under light ether anesthesia. Hot plate response latencies and responses to electric footshock were determined on one occasion only for each type of test. Responses to footshock: 0 = no response; F = flinch response; R = other response.

Rats with DOCA-Salt Hypertension

Experiment 1

Hot plate latencies measured at weekly intervals from the 5th week after implantation were not different, although pronounced hypertension was present (table 1). Electric footshock responses did not differ in hypertensive and sham-operated rats as assessed 8 weeks following surgery (table 2).

Experiment 2

In these animals blood pressure was measured under light ether anesthesia 4½ weeks after implantation. In the DOCA-salt treated animals, systolic blood pressure was significantly higher than in the sham-operated rats. Neither with the hot plate test performed on the 34th day after surgery or in the electric footshock paradigm (36th postoperative day) was a difference in pain sensitivity observed. Blood pressure values obtained after these tests had been performed were similar to those determined before experimentation. Results are summarized in table 3.

Discussion

Our data indicate a diminished pain sensitivity in both young and adult SHR as compared to normotensive WKY controls, which is in agreement with reports by Zamir et al. and Saavedra. The reduction of the hot plate response latency time in SHR to the level of that observed in WKY by pretreatment with the opiate antagonist naloxone points to the involvement of opioid peptides in this respect. The SHR showed a significantly greater number of "no responses" than the WKY in the electric footshock test, indicating an elevated pain threshold in the SHR and confirming the hot plate results. However, the number of flinch responses was smaller in the SHR than in the WKY, which may be due to the general hyperreactivity of the rats from this strain. SHR are probably more inclined to an "exaggerated" response to noxious stimuli than WKY. In the hot plate experiments, SHR showed an increased tendency to jump rather than paw licking or jerking as compared to WKY, pointing to the same hyperreactivity.

The blood pressure of 4-week-old SHR is not different from that of WKY as shown in figure 2. However, at this age when the animals were tested for the first time, the difference in pain sensitivity as measured with the hot plate method is clearly present, confirming the observations of Saavedra. Apparently, a direct correlation between the blood pressure level and responsiveness to noxious stimuli does not exist.

Despite hypertension in the DOCA-salt treated rats that was as severe as in the SHR, no change in pain sensitivity in the former group was observed. The dissociation between hypertension and pain sensitivity was further substantiated by the observation that SHR, in which the development of hypertension was partly prevented by captopril or hydralazine treatment, showed a hot plate response latency that was not significantly different from that observed in hypertensive control SHR.

With neither method used for the assessment of pain sensitivity was a change in responsiveness to noxious stimulation observed in both the renal and the DOCA-salt hypertensive animals as compared to controls, although in both groups of rats severe hypertension had developed. These observations differ from the findings of Zamir et al. in rats of the SABRA strain. We have no explanation for the discrepancy between the present data in renal and DOCA-salt hypertensive rats and the results of Zamir et al. However, as shown in figure 2 and tables 1, 2, and 3, blood pressure levels do not seem to be the cause. In addition, the induction of renal hypertension in WKY rats did not change their hot plate response latency times, therefore, the high blood pressure per se is unlikely to be the cause of the decreased pain sensitivity in SHR (results not shown).

Our initial experimental protocols were similar to those described by Zamir et al. and Zamir and Segal, in which hot plate response latencies were determined repeatedly. It has been reported, however, that repeat-
ed exposure may affect responsiveness to thermal stimulation. Therefore, further experiments were performed in which both renal and DOCA-salt hypertensive rats and the appropriate controls were tested on the hot plate on one occasion only. No difference in hot plate response latency times between hypertensive and normotensive animals were observed, confirming the results of the initial experiments. Similar observations were made in the electric footshock paradigm (table 3).

Since the opiate antagonist naloxone reduces the prolonged hot plate response latencies in SHR to the level of WKY without influencing the response in the latter animals, endogenous opioid peptides apparently play a role in the decreased pain sensitivity of SHR. However, involvement of these peptides in the genesis of hypertension is less clear. Acute administration of naloxone (1 mg/kg, s.c.) does not cause changes in blood pressure in SHR (Sitsen and de Jong, unpublished observations).

Interestingly, the hypnotic action of clonidine and alpha-methyldopa can be antagonized by the opiate antagonists naloxone and naltrexone in SHR but not in WKY. The possible involvement of opioid peptides in the effects of clonidine in SHR was substantiated by the observation, that clonidine releases a beta-endorphin-like immunoreactivity from brain stem slices of SHR but not WKY. Further differences between SHR and other models of experimental hypertension are apparent from the differential antihypertensive effects of clonidine in SHR as compared to rats with renal and DOCA-salt hypertension. Daily administration of clonidine (0.1 mg/kg, orally) for 5 days resulted in a significantly greater fall in blood pressure in SHR than in renal and DOCA-salt hypertensive rats. These findings indicate a response to central alpha-adrenoceptor stimulation involving opioid peptides peculiar to SHR but not to WKY, renal, and DOCA-salt hypertensive animals.

In summary, our data provide evidence for a diminished pain perception in SHR, which does not appear to be directly related to their elevated blood pressure. An unaltered pain sensitivity was observed in two other models of experimental hypertension. Consequently, it is suggested that the diminished pain sensitivity in SHR is more likely to result from genetic factors than from hypertension.

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