Hypoalgesia and hyperalgesia with inherited hypertension in the rat

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MANY STUDIES INDICATE THAT blood pressure control systems can attenuate the processing of brief noceptive signals (9, 33, 34). For example, the spontaneously hypertensive rat (SHR), which is an important model of human primary (genetic) hypertension, exhibits longer noceptive latencies than its normotensive Wistar-Kyoto (WKY) control in the hot plate test (15, 23, 38, 39, 56), tail-flick test (37, 47), and Randall-Siletto paw pressure tests (3). Similarly, hypertensive humans have higher pain thresholds and smaller pain responses in tests that involve acute noxious stimuli (1, 10, 55). Other studies comparing normotensive and hypertensive animals, however, indicate that the direction of this change in noceptive processing is variable. In some studies, adult WKY rats and SHRs did not differ in the tail-flick test (26) or hot plate test (51, 53), and in studies of persistent noception using the Formalin test, SHR exhibited normal (47) or exaggerated noceptive responses (45). To test the hypothesis that qualitative differences in noception between SHR and normotensive rats vary in direction with the pain test used, in the present studies, we compared noceptive responses in SHRs and two normotensive control strains in multiple models of acute noception (hot plate, tail-flick, and paw-flick tests) as well as in two models of persistent inflammatory pain (zymosan and Formalin tests). Indeed, although the number of people whom develop chronic pain and/or hypertension is large, few studies have addressed the contribution of hypertension to the development and maintenance of persistent pain.

With the use of two models of secondary (experimental) hypertension, Sitsen and de Jong (38, 39) found no association between blood pressure and hot plate latency and suggested that the high noceptive thresholds in SHRs resulted from genetic factors rather than from sustained elevations in arterial pressure. To test this hypothesis in the present studies, we evaluated noceptive responses in a different genetic model of primary hypertension, the Dahl salt-sensitive (SS) rat. This rat, but not its control, responds to daily administration of a high-salt diet and/or Wistar-Kyoto controls, SHR were 1) hypoalgesic in the hot plate test and 2) hyperalgesic in longer latency tail and paw-withdrawal tests and in two models of inflammatory nociception. These differences were not observed between SS and salt-resistant controls fed a high-salt diet. Inflammatory hyperalgesia in SHR was correlated with neither paw edema nor the number of Fos-positive spinal cord neurons. Our results indicate that “pain” phenotype of the SHR is not restricted to hypoalgesia. This phenotype is related to genetic factors or to the autonomic systems that control blood pressure and not to sustained elevations in blood pressure, differences in spinal neuron activity, or inflammatory edema.

With the use of two models of secondary (experimental) hypertension, Sitsen and de Jong (38, 39) found no association between blood pressure and hot plate latency and suggested that the high noceptive thresholds in SHRs resulted from genetic factors rather than from sustained elevations in arterial pressure. To test this hypothesis in the present studies, we evaluated noceptive responses in a different genetic model of primary hypertension, the Dahl salt-sensitive (SS) rat. This rat, but not its control, responds to daily administration of a high-salt diet and the rapid development of hypertension. Importantly, because different selection criteria [i.e., development of spontaneous hypertension in SHR (30) vs. hypertension induced by dietary salt in SS rats (41)] were used to derive the original strains, it is likely that the genes that underlie the development of hypertension in the SHRs and SS rats differ. If sustained elevations in blood pressure contribute to altered noception in the SHR, then SS rats and SHRs should exhibit similar noceptive responses compared with their normotensive controls.
METHODS

Animals

Male WKY, SHR, and Sprague-Dawley (SD) rats were obtained from Charles River Laboratories (Hollister, CA). Inbred SS and salt-resistant (SR) rats were originally obtained by Dr. John Rapp [Medical College of Ohio (36)] and Harlan Sprague Dawley (Indianapolis), respectively, and then subsequently bred at the University of California San Francisco (UCSF) animal facilities. Several days before surgery or testing, animals, weight matched at 270–330 g, were individually housed in standard clear plastic cages in a light-dark cycle (6 AM lights on) with food and water provided ad libitum. SS and SR rats were fed a high-salt diet (2%) for at least 7 days before assessment of nociceptive responses. The Institutional Animal Care and Use Committee of UCSF approved all of the following protocols.

Surgical Procedures

Arterial catheterization. We constructed femoral arterial catheters by heat fusing a 4.5-cm length of polyethylene (PE)-10 tubing to a 14.5-cm length of PE-50 tubing. While the rats were under pentobarbital sodium anesthesia (50–60 mg/kg), we isolated the left femoral artery by blunt dissection with care taken not to injure the femoral vein or sciatic nerve. Through a small slit cut into the vessel, we advanced the catheter, prefilled with 100 IU/ml heparin, proximally to the renal bifurcation of the abdominal aorta. After securing the catheter to the vessel with 4–0 sutures, we tunneled the PE-50 end of the catheter under the skin, exteriorized it at the nape, and sutured it to the dorsal neck muscles (splenicus cervicus) (42). After the rats recovered from anesthesia, we returned animals to their cages and allowed them to recover for 3–5 days before testing.

Behavioral Assessment of Nociceptive Reflexes in Naive and/or Zymosan-Treated Rats

Hot plate test. One day before testing, animals were acclimated to the unheated hot plate twice for 1 min. Rats were placed on the hot plate (52.5°C) until they licked their hind paw or exhibited escape (jumping) behavior. Two measurements, taken 1 h apart, were averaged.

Thermal threshold. Tail-flick latency was measured using a technique modified from Hargreaves et al. (13). Briefly, after the tail was blackened with ink (to reduce response latency), rats were acclimated to a clear Plexiglas box on a glass floor for at least 1 h. At specified time points, the stimulus was applied once to each of four designated bands of the tail, 1 in. (2.2 cm) apart. The average of the measurements was calculated. If the rat did not respond within 15.0 s, the heat was terminated to prevent tissue damage. Paw withdrawal latency was measured, as previously described (44), with a cut-off latency of 20 s. Short-latency tail flick, long-latency tail flick, and paw withdrawal were defined by latencies of −4 ± 1.0, 7 ± 1.0, and 10 ± 1.0 s, respectively, achieved by adjusting the voltage intensity of the light bulb.

Mechanical threshold. After 1 h of acclimation to Plexiglass cages with a wire mesh bottom, mechanical threshold for paw withdrawal was assessed using the up-down method modified by Chaplan et al. (2). Thresholds were determined by applying calibrated von Frey hairs (Stoelting, Wood Dale, IL), numbers 3.61–5.18, to the center of the plantar hindpaw with sufficient force to produce slight bending. First, an intermediate von Frey hair was applied. If a brisk withdrawal response occurred within 8 s, the next weaker hair was applied. In the absence of a response, the next stronger hair was applied. This continued until the stimulus threshold could be determined as follows: 50% g threshold = \(10^{(X-\mu)/\sigma} \times 10^k\), where \(X\) is the value (in log units) of the final von Frey hair used, \(\mu\) is the tabular value for the pattern of positive/negative responses, and \(\sigma\) is the mean difference (in log units) between von Frey hairs.

Zymosan-induced nociception. After the subcutaneous injection of zymosan (2 mg in 100 μl) into the midplantar paw, thermal and tactile paw-withdrawal thresholds were assessed as above. Each animal was used only once, i.e., zymosan injection was never repeated in the same animal.

Formalin Nociception

Each animal was transferred to a bedded 10 × 10 × 10-in. Plexiglas box in the laboratory, with food and water provided ad libitum, at least 16 h before testing. Such adaptation to the test environment decreases the variability associated with behavioral measurement in the Formalin test [Tjolsen et al. (48)]. After this acclimation period, we connected the arterial catheter to a pressure transducer (Kobe, Arvada) with PE-50 tubing.

Cardiovascular recording. We began cardiovascular recording at least 20 min later; this time period allows blood pressure and heart rate (HR) in the awake animal to reach resting state (42). Mean arterial pressure (MAP) and HR measurements were recorded once per minute. Resting MAP and HR were calculated as the mean of five measurements collected just before the injection of Formalin. For the analysis of Formalin-induced increases in MAP and HR, the time intervals 1–5 were combined to yield phase 1, 11–15 were combined to yield the interphase, and 21–70 were combined to yield phase 2.

Behavioral assessment. The Formalin stimulus consisted of a 50-μl sc injection of Formalin [37% (wt/wt) formaldehyde, diluted to 1.25% in saline] into the midplantar region of the right hindpaw. Formalin-induced flinching and licking responses, both shown to be reliable measures of the central transmission of nociceptive signals in the Formalin test [Tjolsen et al. (48)], were evaluated. To quantify pain behavior during phase 1, the number of flinches or the number of seconds spent licking during the second, third, fourth, and fifth minute after injection were counted as previously described [Taylor et al. (44)]. From 8 to 90 min, flinches and time spent licking were counted for 2 min at 5-min intervals. These numbers were divided by two, yielding values per minute. With this method, behavior in two animals was simultaneously recorded by one observer at 1–2, 2–3, 3–4, 4–5, and then 8–10, 13–15, . . . , 68–70 min after the Formalin injection. Each animal was used only once, i.e., Formalin injection was never repeated in the same animal.

Evaluation of Edema

While the rat was gently restrained, paw thickness was measured with a Mitutoyo pocket gauge microcaliper (Western Tool, Oakland, CA) as previously described (32). Three measurements were taken and averaged. In the zymosan studies, paw thickness was determined immediately after the measurement of mechanical threshold/thermal latency. This minimized the possible effect of restraint on behavioral responses. In the Formalin studies, paw thickness was measured 70 min after intraplantar injection.
**Immunocytochemistry**

Two hours after Formalin injection, each rat was deeply anesthetized with pentobarbital sodium (100 mg/kg ip) and intracardially perfused with 100 ml of 0.1 M PBS (pH 7.4) followed by 500 ml of 10% Formalin in 0.1 M phosphate buffer. Next, the brain and lumbar spinal cord were removed, postfixed for an additional 4 h, and then cryoprotected overnight in 30% sucrose in 0.1 M PBS. Forty-micrometer frozen sections were cut in the transverse plane and collected in 0.05 M PBS. The sections were then washed with a solution of 0.05 M PBS, 1% normal goat serum, and 0.3% Triton X-100, incubated for 1 h at room temperature in 0.05 M PBS, 5% normal goat serum, and 0.3% Triton X-100. Sections were incubated for 40 h at room temperature in a rabbit anti-Fos antibody (kindly provided by Dr. Dennis Slamon, Univ. of California Los Angeles), as previously described (31). This antibody was diluted 1:21,000 and preabsorbed against aceto-dried liver powder for 1 h at 37°C and for 1 h at 4°C before use. The sections were again washed and incubated in biotinylated goat anti-rabbit IgG and avidin-biotin-peroxidase complex [method adapted from Hsu et al. (16)]. To visualize the immunoreaction product, we used a nickel-intensified diaminobenzidine protocol with a glucose-oxidase reaction [adapted from Llewellyn-Smith and Minson (21)]. Immunoreacted sections were mounted onto slides and then placed under a coverslip using Eukitt mounting medium (Calibrated Instruments, Hawthorne, NY). To quantify the number of Fos-like immunoreactive (Fos-LI) neurons, we counted labeled neurons in each of the four to six photographs per treatment, one investigator manually counted the number of Fos-LI neurons, and then repeated the count from a different investigator. The number of Fos-LI neurons was averaged for the statistical analysis.

**Paw Skin Temperature**

In naïve rats, we assessed paw-surface temperature with a contact surface probe (model 427, Yellow Springs Instruments) and thermometer (model 4600, Yellow Springs Instruments). After the paw was extended, the probe was placed on the skin at the center of the plantar surface. We allowed 60 s for the skin temperature to reach steady state.

**Data Analysis and Statistics**

One-way ANOVA was used to analyze thermal and tactile thresholds in response to plantar injection. Two-way ANOVA with strain and time as the between-subjects variables was used to analyze the responses to Formalin injection. Two-way ANOVA with strain and laminae as the between-subjects variables was used to analyze Fos expression after Formalin injection. If significant, these analyses were followed by appropriate one-way ANOVAs and/or post hoc tests.

**Materials**

Stock solutions of Formalin [aqueous solution of 37% (wt/wt) formaldehyde, Fisher, Fair Lawn, NJ] were diluted in 0.9% isotonic saline (Baxter Healthcare, Deerfield, IL). Pentobarbital sodium was obtained from Abbott Laboratories (North Chicago, IL). The avidin-biotin-peroxidase complex was obtained from Vector Labs (Burlingame, CA). Zymosan was obtained from Sigma (St. Louis, MO).

**RESULTS**

**Resting Blood Pressure, HR, and Paw-Surface Temperature**

To demonstrate that blood pressure was indeed elevated in the SHR and SS models of genetic hypertension, we used indwelling arterial catheters to evaluate baseline MAP and HR in these strains and in their controls. As shown in Fig. 1, resting arterial blood pressure was significantly greater in SHRs compared with WKY or SD controls (P < 0.05) and significantly greater in SS rats compared with SR rats (P < 0.05). Resting HR was greater in SD and SS rats compared with the other strains.

Because sympathetic tone is higher in SHRs, it is possible that differences in local temperature could confound results in thermal withdrawal tests. Local temperature of the extremities can significantly affect reflex responses to thermal stimuli, as in the tail-flick test. However, paw skin temperature in SHRs (27.0 ± 0.8°C, n = 6) was not significantly different from that of WKY rats (27.7 ± 0.7°C, n = 6, P > 0.05).

**Transient Tactile and Thermal Reflexes in SHR, WKY, and SD Rats**

To test the hypothesis that differences between SHR and normotensive rats vary with the type of acute thermal pain test, we compared their responses in the hot plate test, in the short-latency thermal tail-flick test (n = 16–30), and in two long-latency thermal tests.
(tail flick and paw withdrawal). To compare responses to a tactile stimulus, we also evaluated paw-withdrawal responses to von Frey filaments. Figure 2A illustrates that hot plate latencies were longest in the SHR. One-way ANOVA revealed a significant effect of strain \( F(2,47) = 29.2, P < 0.0001 \). Both short-latency tail-flick responses [Fig. 2B, \( F(2,72) = 2.6, P < 0.05 \)] and longer latency responses [Fig. 2C, \( F(2,47) = 7.9, P < 0.005 \)] elicited from the tail were shortest in SHR. Similarly, thermal responses elicited from the paw were shortest in SHR [Fig. 2D, \( F(2,47) = 10.5, P < 0.0005 \)]. ANOVA of mechanical thresholds revealed a significant effect of strain \( F(2,47) = 13.5, P < 0.0001 \). Figure 2E illustrates a dissociation between blood pressure and von Frey thresholds that can be rank ordered as follows: SD > SHR > WKY.

**Persistent Nociceptive Responses in the Setting of Inflammation in SHRs**

**Zymosan.** To test the hypothesis that hypertension is associated with altered nociceptive responses in the setting of inflammation, we evaluated acute tactile and thermal nociceptive responses in awake, unrestrained rats after the injection of zymosan. Zymosan is the active inflammatory substance of brewer’s yeast (*Saccharomyces cerevisiae*). Unlike carrageenan, which predominantly decreases thermal thresholds (Taylor and Basbaum, unpublished observations), and complete Freund’s adjuvant, which predominantly reduces mechanical thresholds, zymosan has been shown to robustly decrease both thermal and mechanical thresholds (25, 35). Because baseline responses were different between the strains (as shown in Fig. 2), we describe the data not only in terms of actual latencies/thresholds, but also as percent change from baseline.

**Thermal hyperalgesia.** As illustrated in Fig. 3 (top), zymosan decreased thermal latencies to a greater extent in the SHR and SD strains than in the WKY strain. This was true at 2.5 h after injection, whether latency values or percent changes were analyzed. ANOVA of thermal latencies over time revealed a significant effect of strain \( F(2,119) = 11.1, P < 0.0001 \).

**Tactile hyperalgesia.** Figure 3 (middle) illustrates that zymosan decreased tactile thresholds to a greater extent in the SHR strain than in the WKY strain. This was true at 1.5 h after injection, whether gram threshold values or percent changes were analyzed. When analyzed as a percentage of baseline, both SHR and SD rats displayed a greater decrease in tactile threshold than did WKY rats. ANOVA of von Frey thresholds over time revealed a significant effect of strain \( F(2,85) = 3.8, P < 0.05 \).

**Edema.** As noted above, hypertensive animals characteristically demonstrate heightened levels of sympathetic activity. Because sympathetic activity modulates inflammation, (17, 41), we considered the possibility that differences in inflammatory responses contributed to any differences in nociceptive responses. To test this hypothesis, we compared zymosan- and Formalin-induced edema in SHRs and their normotensive controls. As shown in Fig.
3 (bottom), SHRs exhibited less edema than either WKY or SD rats at the earlier time points. These differences resolved within 4 h of injection. ANOVA of paw thickness over time revealed a significant effect of strain \( F(2,119) = 7.7, P < 0.0001 \).

**Formalin.** In this model, the intraplantar injection of dilute Formalin first produces a rapid-onset, short-lived phase of painlike behavior (phase 1). An intermediary quiescent period of 10–15 min is then followed by a longer, persistent phase (phase 2) (6, 48). We previously reported that SHRs exhibit exaggerated flinching behavior and cardiovascular nociceptive responses during both phases of the Formalin test. Because of reports that the WKY strain is not genetically consistent from vendor to vendor (19), we compared SHRs not only with WKY rats, but also with outbred, normotensive SD rats. Furthermore, to determine whether differences in spinal nociceptive processing contribute to these observations, we compared not only behavioral responses, but also paw thickness and the expression of spinal cord Fos-LI in SHRs and normotensive rats.

**Nociceptive responses.** As illustrated in Fig. 4A, 1.5% Formalin produced exaggerated flinching responses in the SHR compared with WKY or SD rats during phase 2. Phase 1 flinching responses did not differ. ANOVA of flinching responses over time revealed a significant effect of strain \( F(2,410) = 6.0, P < 0.0001 \). As illustrated in Fig. 4B, 1.5% Formalin produced exaggerated MAP responses in the SHR compared with the WKY or SD rats during phase 2. Phase 1 MAP responses were greater in the SD rats compared with the WKY rats. ANOVA of blood pressure responses over time revealed a significant effect of strain \( F(2,252) = 16.1, P < 0.0001 \).

**Spinal cord Fos expression in WKY, SHR, and SD rats.** Figure 4C illustrates that the number of Formalin-induced Fos-LI neurons in laminae I-II of the lumbar spinal cord was greatest in SD rats, whereas the number of neurons in laminae III-IV was fewest in the SD rats. Fos expression in other laminae of the dorsal horn did not differ.

**Edema.** As illustrated in Fig. 4D, Formalin-induced edema was smaller in the SHRs compared with the WKY rats. Edema in SD rats was not statistically smaller than in WKY rats. ANOVA of paw-thickness responses over time revealed a significant effect of strain \( F(2,47) = 3.9, P < 0.05 \).

**Acute and Persistent Nociceptive Responses in SS Hypertensive Rats**

With the use of a different genetic model of primary hypertension, the SS rat, we next tested the hypothesis...
that sustained elevations in arterial pressure contribute to differences in nociception between normotensive and SHRs. Figure 1 demonstrates that 7 days of a high-salt diet led to elevated MAP in SS but not in control SR rats. Figure 5 illustrates that neither hot plate, tactile, nor tail-flick latencies differed between the SS and SR strains whether they were tested before or after salt administration (P > 0.05). As illustrated in Fig. 6 (top), SR rats showed only a modest difference in flinching responses during phase 1 compared with SS rats. Figure 6 (bottom) illustrates that SS rats exhibited smaller phase 2 MAP responses than SR rats. ANOVA of blood pressure responses across time revealed a significant effect of strain \[F(1,202) = 14.3, P < 0.0001\].

**DISCUSSION**

**Hypoalgesia and Hyperalgesia in the SHR**

An important conclusion from this study is that the nociception phenotype of the SHR is not primarily one of hypoalgesia. Rather, the SHR phenotype varies with the nociceptive test. Compared with normotensive WKY controls, SHRs were hypoalgesic in the hot plate test and hyperalgesic in tail- and paw-withdrawal tests.
and in the Formalin and zymosan models of inflammatory nociception. Because of evidence for genetic heterogeneity in the WKY rat control strain (19), we also compared SHRs to outbred SD rats and again found the SHR to be hypoalgesic in the hot plate test, not different in the short-latency tail-flick test, and hyperalgesic in longer latency tail- and paw-withdrawal tests and in the Formalin model of inflammatory nociception. We conclude that hypoalgesia in the SHR is restricted to a limited number of acute pain models such as the hot plate test.

There are several possible explanations for our observation that the SHR phenotype varies with the nociceptive test. First, the genetic determinants of behavioral responses to noxious stimuli in SHR and normotensive rats may vary with the pain assay (7, 27, 28). In fact, the genetic mechanisms involved in the persistent inflammatory nociception of the Formalin and zymosan models are likely quite different from those involved in acute nociception (29). Second, because the orienting response that is associated with enhanced sensory processing (11, 40) is blunted in SHRs (43, 46), SHRs may be less able to appropriately adapt to the hot plate stimulus, leading to a dampened response. Third, the differences between SHR phenotype within the thermal modality may be related to the requirement for animal handling just before the hot plate test but not before the application of the thermal stimulus in the Hargreaves’ test.

Because the fight-or-flight stress and sympathetic responses are exaggerated in SHRs (24, 43, 50) and these responses have been associated with hypoalgesia, it is possible that the stress-induced analgesia (SIA) associated with the hot plate test is enhanced in SHRs, leading to a dampened response. In contrast to previous studies that used more traditional behavioral pain assays in restrained animals (3, 15, 23, 37–39, 47, 56), the current study evaluated paw- and tail-withdrawal latencies in the unrestrained rat. Because SIA might alter the behavioral phenotype of acute nociception in the SHR, we suggest that the differential response to stress is a major contributor to the different results obtained in the present and previous studies.

Nociception and Sustained Elevations in Arterial Pressure

By comparing SHR and SS rats with their respective controls, we found that the abnormal nociceptive responses in the SHR are related to mechanisms other than sustained elevations in blood pressure. Although the high-salt diet increased blood pressure in SS rats to a level comparable with that of SHRs, the differences in acute nociception observed in SHR, WKY, and SD rats were not found in the SS and SR rats. By contrast, with the use of older models of acute nociception, Friedman et al. (8) found that SS rats, compared with SR rats, exhibited longer tail-flick latencies during animal restraint and higher flinch-jump thresholds to electric shock. However, the differences in baseline responses observed before salt administration confound interpretation of those results.

The present studies demonstrate that the exaggerated Formalin-evoked responses in SHRs were not observed in SS rats. In fact, the SS rats displayed smaller blood pressure responses compared with control, which could merely be due to a ceiling effect associated with the high resting blood pressure in this strain. A decoupling of blood pressure from nociception was also observed by Sitsen and de Jong (38, 39), who reported that experimentally induced hypertension did not produce increases in hot plate latency, by Tsai and Lin (49), who reported that SHRs exhibited longer hot plate latencies at 30 days of age, before the development of a robust hypertension, and by Ghione et al. (10), who found that pharmacological reduction of blood pressure in hypertensive humans did not alter their pain sensitivity.

Although zymosan hyperalgesia was greater in the SHR compared with the WKY rat, it was not different between the SHR and the SD rat. This makes it difficult to interpret the results with respect to differences in resting blood pressure. Similarly, blood pressure differences could not explain the results of Wiesenfeld-Hallin and colleagues, who found that SHR, WKY, and SD rats exhibit widely different painlike behaviors and primary afferent discharge after nerve injury (12, 22, 54). Rather, they suggested that a genetic predisposition unrelated to high blood pressure accounts for much of the variability in neuropathic pain behavior.
across different strains of mice (28), rats (5), or possibly humans.

We cannot rule out the possibility that these interstrain differences derive, in part, from an interaction of genotype and hypertension. For example, because the hypertension in the SHR existed for a much longer time (~8 wk) than the SS rat (~10 days), it is possible that long-term compensatory mechanisms in the SHR, but not the SS rat, include alterations in nociceptive pathways. The bulk of the evidence, however, suggests that blood pressure per se is not the primary factor mediating differences in nociception between SHR and normotensive rats.

**Persistent Nociception in SHRs**

In the zymosan test, we found SHRs to be hyperalgesic compared with their inbred normotensive WKY controls. Our results do not agree with Chipkin and Latranyi (3), who found that the intraplantar injection of yeast (the compound from which zymosan is purified) decreased paw pressure threshold in WKY and SD rats but increased paw pressure threshold in SHRs. Possible explanations for this discrepancy include 1) the use of paw pressure threshold in the earlier study versus the use of thermal and von Frey thresholds in our study; 2) the possible existence of a contaminant in yeast (other than zymosan) that produced antinociception in the SHR but not the WKY or SD rats; or 3) measurement of behavior in the earlier study at a single, 1-h time point, before peak changes in nociceptive threshold could be reached. Indeed, we found that SHRs exhibit hyperalgesia 1.5–2.5 h after intraplantar injection.

To further evaluate inflammatory hyperalgesia, we studied persistent nociception in a second model, the Formalin test. We found exaggerated behavioral and cardiovascular responses to Formalin during phase 2 in the SHR. This did not correlate with an increase in the number of Formalin-induced Fos-positive neurons in the spinal cord dorsal horn or with an increase in Formalin-induced edema in the paw. On the contrary, paw edema was smaller in the SHR than in the WKY rat and, in the case of zymosan, was smaller in the SHR than in either normotensive strain. One possible explanation is that glucocorticoid-mediated inhibition of inflammation is exaggerated in the SHR. In fact, plasma levels of cortisol and corticosterone are greater in SHRs (14, 18) and in humans predisposed to hypertension (52), respectively.

We conclude that mechanisms other than spinal nociceptive transmission and inflammatory edema contribute to exaggerated Formalin-induced behavioral and cardiovascular responses in the SHR. Because sympathetic activity is positively coupled to inflammatory hyperalgesia (17), it remains possible that exaggerated sympathetic activity in the SHR increases the development of peripheral sensitization mechanisms. Such a mechanism may accelerate the development of adjuvant-induced arthritis in SHRs (20). Future studies that evaluate the development of peripheral sensitization in the SHR are needed.

In summary, our results indicate that the pain phenotype of the SHR is not restricted to hypoalgesia. Rather, differences in behavioral responses to noxious stimuli between SHR and normotensive rats vary with the nociceptive test. SHR hyperalgesia appears to be related not to sustained elevations in blood pressure per se, spinal neuron activity, or inflammatory edema, but rather may be related to genetic factors or to autonomic systems that control blood pressure. If certain genes pleiotropically affect both hypertension and nociception, then quantitative trait locus mapping techniques of loci involved both in pain (28) and hypertension (36) could yield valuable information regarding differential nociceptive processing in hypertensive individuals.

**Perspectives**

Although the number of people who develop chronic pain and/or hypertension is large, few studies have addressed the contribution of hypertension to pain threshold. Fewer, if any, studies have evaluated nociception in hypertensives with chronic debilitating pain states. A limited number of animal and clinical studies have found that hypertensive subjects show increased pain threshold to acute noxious stimulation, but we suggest here that these results may be confounded by stress-induced analgesia. Indeed, when we minimized stress with models of acute nociception in unrestrained animals and more clinically relevant models of persistent nociception and inflammatory hyperalgesia, we observed exaggerated nociceptive responses in the SHR. Further animal studies as well as measurement of persistent pain in patients with hypertension will facilitate our understanding of how these factors contribute to persistent pain.

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