Does So-called Streptozocin Hypertension Exist in Rats?

MIHO KUSAKA, KOICHIRO KISHI, AND HIROFUMI SOKABE

SUMMARY Although the existence of so-called streptozocin hypertension seems well established, some reports have indicated that no rise in blood pressure (BP) occurred after streptozocin treatments. To ascertain the streptozocin-induced BP response, normotensive Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) were treated with streptozocin, 40 to 45 and 35 mg/kg i.v., respectively, and BP was determined directly and indirectly every week for 3 to 4 weeks. Direct mean BP was determined without anesthesia or restraint through a cannula inserted into the rat's abdominal aorta. Indirect BP was determined at the tail without anesthesia after prewarming the rat in a holder. Compared with control values, indirect BP increased significantly in diabetic WKY 2 weeks after streptozocin treatment. In contrast, direct BP of these rats decreased, compared with control values. Indirect BP of diabetic SHR was as high as that of the controls, whereas direct BP of diabetic SHR decreased significantly 1 week after the treatment and thereafter, compared with control values. These discrepancies between the direct and indirect BP values may be caused by severe emaciation of diabetic rats. Extra pressure in the cuff may be necessary to occlude the bloodstream. These results indicate that under these conditions the value of BP obtained by the direct measurement is more reliable than that by the indirect one; therefore, we concluded that so-called streptozocin hypertension does not exist. (Hypertension 10: 517–521, 1987)

KEY WORDS • spontaneously hypertensive rats • Wistar-Kyoto rats • streptozocin

S O-CALLED streptozocin (STZ) hypertension in rats seems to have become a well-established form of experimental hypertension since its demonstration in Sprague-Dawley rats in 1978 by Kawashima et al.1 However, a survey of the literature indicates controversy as to the development of hypertension after STZ treatment. STZ was reported to induce not only diabetes2 but also hypertension in Wistar or Sprague-Dawley rats,1,3,4 and several studies have attempted to investigate the mechanism of the development of hypertension induced by STZ diabetes.3,5,6 Alterations of the prostaglandin and/or the kallikrein-kinin systems, impaired renal prostaglandin E2 synthesis, and altered hypothalamic pressor responses have been suggested to play a role in the development of hypertension in STZ-induced diabetic rats. On the other hand, some reports do not support the existence of hypertension in STZ-diabetic rats.7,8 Somani et al.9 reported that blood pressure (BP) in Wistar-Kyoto rats (WKY) rises progressively with increasing dose of STZ, whereas STZ induces a dose-dependent decrease of BP in spontaneously hypertensive rats (SHR). Rodgers et al.10 reported that STZ induces a depressor effect in SHR and has no effect in WKY. Of these groups,1-10 only two tried to determine BP directly.3,9 All other groups determined BP using the indirect tail-cuff measurement.

In a previous study, in which we tried to develop an animal model of hypertension with diabetes mellitus, we found a discrepancy between BP values determined by direct cannulation and the indirect tail-cuff method.11 In Wistar rats, WKY, and SHR treated with STZ (30-80 mg/kg i.v.), the indirect BP measurement yielded hypertensive values whereas rather low BP values were obtained by the direct measurement.11 To our knowledge, no previous study has conducted parallel determinations of direct and indirect BP in STZ-
induced diabetic rats. Thus, in the present study, we analyzed the effect of STZ treatment on BP in normotensive and hypertensive rats and we measured BP indirectly by the tail-cuff method and directly through a cannula inserted into the abdominal aorta without anesthesia or restraint, for 3 to 4 weeks after STZ treatment.

**Materials and Methods**

Ten-week-old male WKY and SHR, from the colonies of the Department of Pharmacology, Jichi Medical School, Tochigi-ken, Japan, were used. All rats were fed normal chow (MR-3-A; Nihon Nosan, Yokohama, Japan) and tap water ad libitum. After a 1-week control period, an indwelling cannula was inserted into the abdominal aorta in each rat. Five days after the operation, WKY and SHR were injected intravenously with STZ (40-45 and 35 mg/kg, respectively), through the dorsal tail vein under light ether anesthesia after an overnight fast. At these doses, STZ has been shown to induce marked hyperglycemic effects (blood glucose > 300 mg/dl) in these rats. 9 STZ (Sigma Chemical, St. Louis, MO, USA) was dissolved in 0.9% NaCl solution containing 1 mM citrate buffer (pH 4.5) immediately before use, at a volume of 1 ml/kg body weight. Solvent was injected into the control WKY and SHR. Rats were housed in rat cages in an animal room with constant temperature (25 ± 1 °C) and humidity (60 ± 5%) and a 12-hour light cycle (0700-1900). Body weight was determined once a week.

**Determination of BP and Heart Rate**

BP was determined directly and indirectly once a week for 3 to 4 weeks after STZ treatment. The direct mean BP and heart rate (HR) were determined without anesthesia or restraint through a cannula inserted into the rat’s abdominal aorta. They were recorded by an electronic system, composed of a transducer (Model CP-01; Century Technology, Inglewood, CA, USA), a pressure amplifier (Model PA-011; Star Medical, Tokyo, Japan), and an HR counter (Model HR-001; Star Medical) and a recorder (Model R-50; Rikadenki, Tokyo, Japan). The values were obtained only after the rat had been left undisturbed for at least 15 minutes and BP had stabilized. On the next day, indirect maximum BP and HR were determined by a rat-tail manometer-tachometer system (Model KN-210; Natsume Seisakusho, Tokyo, Japan). Rats were prewarmed for 30 to 60 minutes in rat holders (Model KN-325 [B]; Natsume Seisakusho) placed on a hot plate with a surface temperature of 35°C. Under these conditions, the temperature in the rat holder was 31 ± 1°C and rats were calm and relaxed in the holders. 12 The effective cuff length was 25 mm. We also used a 35-mm cuff and obtained almost the same results as with the shorter one.

During the last week of the experiments, direct mean and indirect tail BP values were also determined simultaneously after prewarming rats. Pulse pressures were determined directly at the abdominal aorta with the rats under ether anesthesia.

**Determination of Blood Glucose**

Blood glucose level was determined once a week for 3 to 4 weeks. A trace of blood was taken from the cannula, and blood glucose level was evaluated using enzymatic test strips (Diatorol; Sanwa Kagaku Kenkyusho, Nagoya, Japan) every week. The accuracy of this method has been checked by comparing results with those obtained using the quantitative glucose-dehydrogenase method.13

**Rat Tail Morphology**

On the last week of the experiments, the rats were killed with ether. Then, 1-cm tail specimens were taken from the part used for the indirect BP measurement, fixed with 10% formalin, embedded in paraffin, and subsequently stained with hematoxylin-eosin for light microscopic and macroscopic examinations.

**Statistical Analysis**

All data were shown as means ± SE. The statistical significance of the differences between means was determined by the Student’s t test.

**Results**

**WKY**

Table 1 shows the values of body weight, blood glucose, direct HR, and indirect HR of WKY in Weeks 0 and 3. A weight gain was observed in nondiabetic rats, whereas one week after STZ treatment, a significant weight loss was observed in diabetic rats, and their growth was retarded significantly thereafter. In diabetic rats blood glucose levels were elevated significantly. Figure 1 shows the course of BP. In diabetic rats, indirect tail BP was elevated after STZ treatment. The value of tail BP in Week 2 was significantly higher than that of the control. However, direct mean BP obtained through a cannula decreased gradually, and a significant decrease was observed in the second week, compared with control values. In the third week, the direct BP value of diabetic WKY was decreased further. Whereas the indirect BP values obtained after prewarming were increased at this time, the direct BP values measured after prewarming were decreased further (from 98 ± 3 to 87 ± 4 mm Hg; n = 14). Directly measured pulse pressures of diabetic WKY were in the range of 28 to 29 mm Hg, indicating that the higher indirect BP values could not be explained by an abnormal increase in the maximum BP.

Figure 2 shows the cross-sectional features of the tail specimens from the nondiabetic and diabetic WKY. Deformation, shorter diameter, reduced muscle bundle mass, and reduced bone mass were most apparent in the diabetic rats, corresponding to their emaciation. These changes consequently resulted in a relative increase in fibrous tissues, such as the ligaments, in the tail.

**SHR**

Table 1 shows the values of body weight, blood glucose, direct HR, and indirect HR of SHR in Weeks 0 and 4. The weight of nondiabetic rats gradually in-
TABLE 1. Effects of Streptozocin Treatment on Body Weight, Blood Glucose, and Heart Rate in WKY and SHR

<table>
<thead>
<tr>
<th>Variable</th>
<th>WKY</th>
<th>SHR</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 3</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>249 ± 5 (20)</td>
<td>280 ± 5 (16)</td>
</tr>
<tr>
<td>STZ treatment</td>
<td>246 ± 5 (23)</td>
<td>228 ± 8* (14)</td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>92 ± 2 (20)</td>
<td>92 ± 3 (16)</td>
</tr>
<tr>
<td>STZ treatment</td>
<td>97 ± 2 (23)</td>
<td>337 ± 21* (14)</td>
</tr>
<tr>
<td>Direct HR (beats/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>335 ± 6 (20)</td>
<td>329 ± 7 (16)</td>
</tr>
<tr>
<td>STZ treatment</td>
<td>346 ± 8 (23)</td>
<td>294 ± 13* (14)</td>
</tr>
<tr>
<td>Indirect HR (beats/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>349 ± 11 (20)</td>
<td>319 ± 10 (16)</td>
</tr>
<tr>
<td>STZ treatment</td>
<td>356 ± 10 (23)</td>
<td>305 ± 9 (14)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Number of rats is in parentheses. STZ = streptozocin; HR = heart rate.
*p < 0.01, t p < 0.05, compared with control group values.

creased, whereas it slowly decreased in diabetic rats and was significantly lower than that of the control throughout the experiment. A significant increase in blood glucose was observed in diabetic rats, and a decrease in HR was observed in diabetic rats both directly and indirectly. Figure 3 shows the course of BP. Indirect tail BP of diabetic rats and controls slowly increased throughout the experiment, whereas direct mean BP decreased significantly in diabetic rats in Week 1 and thereafter, compared with control values. In Week 4 direct mean BP of diabetic SHR was deter-

![Figure 1](http://hyper.ahajournals.org/)

**FIGURE 1.** Effects of streptozocin (STZ) treatment (40-45 mg/kg i.v.) on direct mean BP and indirect tail (maximum) BP in WKY. Vertical bars represent SEM. Initial number of rats in each group is shown in parentheses after the explanation of symbols. Final number of rats in each group is shown in parentheses after the Week 3 value. Rats whose BP became undetectable before the second week because of death or hemaggregation in the cannula were omitted. Rats were treated with STZ or solvent during Week 0. Statistically significant differences compared with the corresponding control data are indicated by single (p < 0.05) and double (p < 0.01) asterisks.

![Figure 2](http://hyper.ahajournals.org/)

**FIGURE 2.** Features of the cross-section of the tail from non-diabetic (A) and diabetic (B) WKY. Specimens were taken from the part used in the indirect BP measurement and magnified at the same ratio (× 7).
In this study we observed an elevation of BP only when the indirect tail-cuff method was used in WKY with STZ-induced diabetes mellitus. In these rats, direct mean BP was suppressed. In diabetic SHR, indirect tail BP increased slowly, as in their controls, whereas a significant reduction of direct mean BP was observed. Indirect tail BP was measured after prewarming, anesthesia, or restraint through a cannula inserted into the rat's abdominal aorta. Direct BP was obtained after the rat had been left undisturbed for at least 15 minutes and BP had stabilized. Therefore, the values of BP determined by the direct measurement were more physiological and reliable than those obtained by the indirect method. Consequently, the present results suggest that STZ treatment reduces BP in normotensive and hypertensive rats and that hypertension is not induced by STZ-induced diabetes. Although the mechanism by which STZ treatment induces depressor effects in WKY and SHR is not clear, it may be associated with a reduction in vascular collagen biosynthesis, plasma vasoactive factors, or vascular reactivity to plasma vasoactive factors.

In these diabetic rats, directly measured pulse pressures were in the range of 28 to 32 mm Hg and were not so high as to induce the large difference between maximum and mean BP. We considered two possible explanations for the high values of tail BP in these diabetic rats: 1) the elevations were induced by the stress of prewarming and restraint or 2) the increases were caused by severe emaciation of the rat tail. In the last week, direct mean and indirect tail BP values were also determined simultaneously after prewarming the rat loosely restrained in a rat holder. This is a routine condition of indirect BP measurement and has been reported to be fairly nonstressful, as shown by the lack of increase in HR in WKY (see Table 1). As prewarming slightly increased mean BP in diabetic SHR, as compared with values determined without prewarming, the discrepancy of the BP values between direct and indirect measurements may be explained partly by the slight stress of prewarming and restraint. SHR have been shown to be susceptible even to such a slight stimulus. On the other hand, mean BP decreased further after prewarming in diabetic WKY; therefore the discrepancy between results obtained by direct and indirect BP measurements was not induced by the slight stress of prewarming and restraint. The reason for the reduction of mean BP after prewarming in diabetic WKY is not known, but it may be due to peripheral vasodilatation.

The possibility remains that emaciation of the tail in the diabetic WKY and SHR resulted in structural changes that required extra pressure above the maximum to occlude the tail artery. As can be seen in Figure 2, a reduction of the skeletal muscular mass caused a relative increase in fibrous tissues such as the ligaments, which acted to prevent complete compression of the arterial lumen, even when the cuff pressure rose above the maximum. Therefore, we conclude that STZ treatment suppresses, rather than elevates, BP in normotensive and hypertensive rats and that so-called STZ hypertension does not exist.

**Discussion**

In this study we observed an elevation of BP only when the indirect tail-cuff method was used in WKY with STZ-induced diabetes mellitus. In these rats, direct mean BP was suppressed. In diabetic SHR, indirect tail BP increased slowly, as in their controls, whereas a significant reduction of direct mean BP was observed. Indirect tail BP was measured after prewarming the unanesthetized rat for 30 to 60 minutes, while it was loosely restrained in a rat holder placed on a hot plate with a surface temperature of 35°C. On the other hand, direct mean BP was determined without prewarming, anesthesia, or restraint through a cannula inserted into the rat's abdominal aorta. Direct BP was obtained after the rat had been left undisturbed for at least 15 minutes and BP had stabilized. Therefore, the values of BP determined by the direct measurement were more physiological and reliable than those obtained by the indirect method. Consequently, the present results suggest that STZ treatment reduces BP in normotensive and hypertensive rats and that hypertension is not induced by STZ-induced diabetes. Although the mechanism by which STZ treatment induces depressor effects in WKY and SHR is not clear, it may be associated with a reduction in vascular collagen biosynthesis, plasma vasoactive factors, or vascular reactivity to plasma vasoactive factors.

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**Acknowledgments**

We are indebted to Ms. Yuko Nakajima for technical assistance and to Ms. Ryoko Takeda for secretarial assistance. We also thank Dr. Kazuhiro Kumei for his assistance in the examination of rat tails.

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Hypertension. 1987;10:517-521
doi: 10.1161/01.HYP.10.5.517

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1987 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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