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Restraint Alters Temperature Responses to Cocaine in Spontaneously Hypertensive Rats

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ISHIZUKA, Y., R. W. ROCKHOLD, B. HOSKINS AND I. K. HO. *Restraint alters temperature responses to cocaine in spontaneously hypertensive rats.* PHARMACOL BIOCHEM BEHAV **37**(4) 773–777, 1990.—The body temperature responses to intraperitoneal (IP) or intravenous (IV) cocaine in spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats were examined under restrained and freely moving conditions. Resting values for rectal temperature (RT), in ambient temperatures of 22–24°C, were significantly ($p<0.01$) higher in SHR than in WKY rats, under both restrained (39.19 ± 0.07 vs. 38.01 ± 0.06°C) and freely moving (39.39 ± 0.08 vs. 38.08 ± 0.06°C) conditions. Resting RT did not differ between restrained and freely moving conditions within a strain. An heterogeneity of RT response to both IV and IP cocaine was expressed by restraint in SHR. The SHR could be divided into animals which demonstrated hyperthermia to cocaine (SHR_H) and those in which RT fell (SHR_L). However, cocaine produced hyperthermia in all freely moving SHR, regardless of the route of administration. The effects of IV cocaine in restrained WKY rats were similar to those in freely moving SHR, whereas IP cocaine decreased RT in all restrained WKY rats. Under conditions of restraint, divergent RT responses to cocaine were demonstrated following IV and IP administration in WKY rats, but not in SHR. These results indicate that restraint stress can significantly modify the body temperature responses to acute cocaine administration in both SHR and WKY rats.

Spontaneously hypertensive rats Cocaine Body temperature Stress

THE spontaneously hypertensive rat (SHR), originally developed by Okamoto and Aoki (11) at Kyoto University in Japan, has been investigated extensively as a model of genetic hypertension. We have recently reported the effects of cocaine on cardiovascular function, thermoregulatory responses and convulsions in SHR and normotensive Wistar-Kyoto (WKY) rats (6). These genetically related rat strains were employed to permit more effective discrimination between individual variables which alter susceptibility to the toxic actions of cocaine. Devenyi (3) has recently emphasized the idiosyncratic nature of the incidence of cocaine-induced toxicity in man. The genetic similarities between SHR and WKY rats facilitate identification of systematic factors which alter sensitivity to the adverse effects of cocaine.

Previously reported studies have demonstrated that transient restraint expresses a significant heterogeneity in the rectal temperature (RT) responses to cocaine administration in SHR (6). The SHR were found to demonstrate either an increase or a reduction in RT following treatment with cocaine. The former were designated as SHR_H, the latter as SHR_L. The differential responses of RT were found to be a useful predictor of the susceptibility to cocaine-induced convulsions, as the SHR_H evidenced an increased sensitivity to cocaine-induced convulsions and a tachycardia which was both greater in magnitude and more prolonged than that observed in SHR_L.

Certain actions of cocaine appear to be altered following administration by different routes. Intravenous injection of cocaine in the rat produced alterations in glucose utilization in the medial prefrontal cortex and nucleus accumbens which were not observed following IP administration of the drug (12). Nomikos and Spyraki (9) reported that the reinforcing properties of cocaine, in a place preference conditioning paradigm, could be demonstrated following IV, but not with IP administration. In addition, restraint stress can modify body temperature responses to cocaine (6).

The purpose of the present experiments was to utilize SHR and WKY rats to examine variations in the RT responses to acute cocaine administration. Differences in responses following administration of cocaine by two routes, IV and IP, under freely moving and restrained conditions were determined. Moreover, the ability of both IV and IP cocaine challenges to serve as predictors of sensitivity to cocaine-induced convulsions was examined under restrained conditions.

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METHOD

General Procedures

Male SHR and WKY rats were purchased from Taconic Farms, Inc. All animals were 12 weeks of age at the start of the experiments. Animals were housed, prior to experimental use, in plastic cages (3–4/cage) under controlled conditions of temperature (22°C), humidity (50–55%) and lighting (0800–2000 light: 2000–0800 dark) and given rat chow (Purina Laboratory Chow) and tap water, ad lib. Surgical procedures were performed under halothane anesthesia (2–4% in medical grade oxygen). A polyethylene catheter was inserted 4 cm into the vena cava (PE-10) by way of the left femoral vein in each rat. This catheter was filled with heparinized saline (10 U/ml), exteriorized at the nape of the neck and sealed until use. Animals were housed subsequently in individual hanging wire cages. Experimental protocols were initiated two days following catheter implantation.

Experiments Under Freely Moving Conditions

Animals were placed in plastic cages ($24 \times 20 \times 46$ cm; 3–4/cage) at an ambient temperature of 23 ± 1°C 2 h before starting RT measurement. Those rats in which cocaine was administered by the IV route were housed singly in a cage during each experiment to avoid chewing of catheters by other animals. Rectal temperature was monitored with a Model BAT-12 (Sensortek, Inc.) electronic thermometer, using lubricated rectal thermistor probes inserted to a depth of 4 cm. Measurements were taken in each rat serially at approximately 15-min intervals. Each sequence consisted of probe insertion, a 30-s waiting period for temperature equilibration, temperature measurement and probe withdrawal. Rectal temperature measurements were conducted following a 5-day period of daily acclimation to RT measurements. The term control temperature refers to the RT in animals following the 2-h acclimation and immediately prior to drug administration.

Experiments Under Restrained Conditions

Rectal temperature measurements were started 2 h following introduction of the animals to the experimental room in an ambient temperature of 23 ± 1°C. Animals were placed in acrylic restraining cages (5.6 cm in diameter, 18 cm in length) and a lubricated rectal thermistor probe was inserted 4 cm and held in position with adhesive tape. Rectal temperature was monitored with a Model BAT-12 (Sensortek, Inc.) electronic thermometer. In this case, the control temperature refers to the RT obtained 25–35 minutes after each subject was placed in an acrylic restraining cage.

Drug Administration

Cocaine hydrochloride (Sigma Chemical Company) was dissolved in 0.9% sodium chloride. The doses of cocaine were 10 mg/kg for IP injection and 1 mg/kg for IV injection.

Determination of Differential Responsiveness of SHR.

Spontaneously hypertensive rats were assigned to one of two groups based on their RT responses to challenge with cocaine (6). In the present experiment, rats were challenged with either 1 mg/kg cocaine IV or 10 mg/kg cocaine IP administered a minimum of 1 week prior to subsequent experimental use. Cocaine challenge was administered while rats were restrained under ambient conditions of 23 ± 1°C. The response of RT was monitored for at least 60 min following challenge. Rats were designated as SHR_H if the maximum change in RT during that period was positive (i.e., a hyperthermic response) and SHR_L if the maximum change in RT was negative (i.e., a lowering of RT).

Measurement of the Time-to-Onset of Convulsions

Spontaneously hypertensive rats, which were divided into subgroups on the basis of RT responses to a challenge dose of cocaine, were used in these experiments. Cocaine hydrochloride (1.25 mg/kg·min) was infused intravenously into restrained animals using a Sage Model 353 pump, and the elapsed time from the start of infusion until the onset of convulsions (Tc) was measured.

Statistical Analysis

The results were subjected to analysis of variance (one- or two-way for appropriate groups). Newman-Keuls tests were performed to differentiate group means if significant interactions were found by analysis of variance.

RESULTS

Resting values for RT in all SHR were significantly higher ($p<0.01$) than in all WKY rats under both restrained (39.19 ± 0.07 vs. 38.01 ± 0.06°C) and freely moving (39.39 ± 0.08 vs. 38.08 ± 0.06°C) conditions, at ambient temperatures of 22–24°C. The resting RT did not differ between restrained and freely moving conditions within a strain.

Saline (0.9% NaCl) vehicle was used as a control. The volume of saline was 0.1 ml/kg for IV cocaine injection and 1 ml/kg for IP cocaine injection. Neither IV nor IP saline injections altered RT in WKY rats under either restrained and freely moving conditions (Fig. 1, Fig. 3). Saline-treated SHR developed gradual decreases in RT under both conditions (Fig. 2, Fig. 3).

When cocaine was intravenously administered to restrained SHR, 3 SHR showed hyperthermia while hypothermia was observed in the remaining 3 SHR. Intraperitoneal cocaine also produced similar divergent effects on RT in restrained SHR, i.e., hyperthermia was demonstrated in 6 SHR while hypothermia was produced in the remaining 10 rats. The rats that developed cocaine-induced hyperthermia and hypothermia were designated as SHR_H and SHR_L, respectively. The changes in hypothermic and hyperthermic responses to cocaine were significantly different from saline control at 30 min after cocaine injection (Fig. 2).

Cocaine administration, both IV and IP, was performed also in restrained WKY rats. Intravenous cocaine produced significant increases in RT at 30 min following drug administration in restrained WKY rats, when compared to that in the saline vehicle group. On the other hand, significant decreases in RT were obtained at 15 min following IP cocaine injection compared to those in the saline vehicle group (Fig. 1).

In contrast, in freely moving SHR and WKY rats, both IV and IP cocaine increased RT significantly above that of control values at 15 min after cocaine injection (Fig. 3). Moreover, the increases in RT produced by IP cocaine injection were greater than IV cocaine-induced hyperthermia, particularly in the SHR (Table 1). Additionally, the magnitude of RT responses to cocaine, relative to saline vehicle controls, tended in all cases to be greater in SHR than in WKY rats (Fig. 3, Table 1). The maximum change in RT following IP cocaine injection was significantly greater than that of all other groups, including SHR, treated by IV cocaine. The absolute maximum changes in RT following IV cocaine injection were slightly, but not significantly, greater in SHR than in WKY rats.

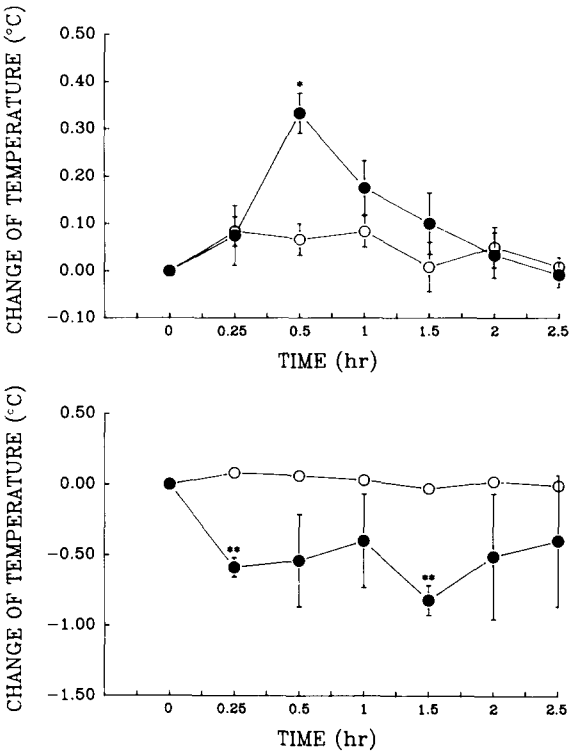

FIG. 1. Rectal temperature responses to IV (upper) and IP (bottom) cocaine in restrained WKY rats. The filled and open circles show data obtained from cocaine- and saline-treated animals, respectively. The vertical lines present the standard errors of the mean. The numbers of animals in each group were, for IV injections: cocaine, 6; saline, 6 and for IP injections: cocaine, 5; saline, 8. Asterisks indicate significant differences ($*p<0.05$, $**p<0.01$) compared to saline-treated groups at corresponding time points.

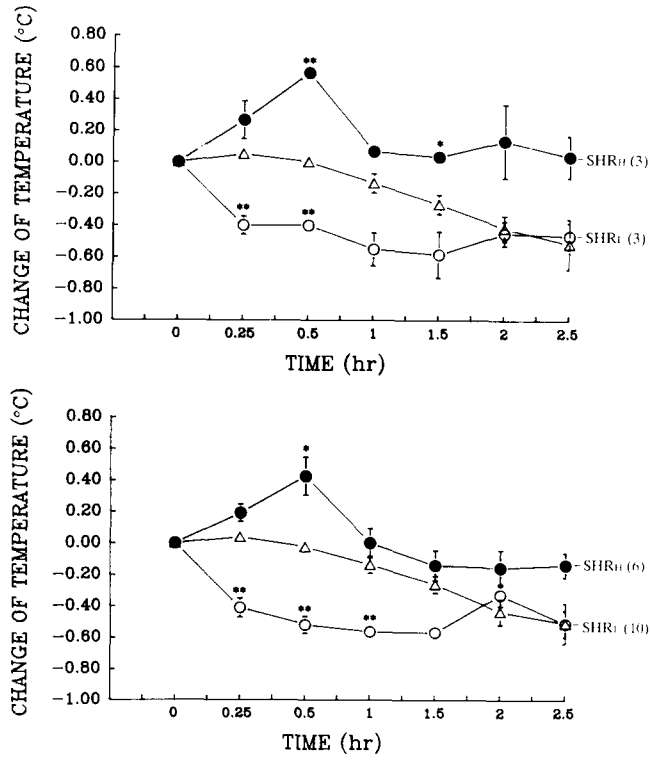

FIG. 2. Rectal temperature responses to IV (upper) and IP (bottom) cocaine in restrained SHR. The circles and triangles show data obtained from cocaine- and saline-treated animals, respectively. The vertical lines represent the standard errors of the mean. Equal numbers of animals received either cocaine or saline vehicle in experiments involving IV (n = 6) and IP (n = 16) routes of administration. Numbers in parentheses indicate the numbers of rats assigned to SHR_H and SHR_L groups. Asterisks indicate significant differences ($*p<0.05$, $**p<0.01$) compared to the saline-treated groups at corresponding time points.

To verify whether the differential responsiveness of RT to cocaine would correlate with sensitivity to cocaine-induced toxicity, SHR were divided into two groups, SHR_H and SHR_L, based on their RT responses to an initial cocaine challenge. One to 2 weeks following this challenge, the elapsed time-to-onset of convulsions (Tc) following cocaine infusion (IV, 1.25 mg/kg·min) was compared between SHR_H and SHR_L. The Tc was found to be significantly shorter in SHR_H than in SHR_L (Table 2). Additionally, it was noted that, in SHR_L grouped following challenge with IP cocaine, the Tc was significantly shorter than that obtained in SHR_L, determined by IV injection of the challenge dose of cocaine (Table 2).

A transient startle response was noted upon IV cocaine injection. Modest increases in locomotor activity and initiation of grooming behaviors were evident in both freely moving SHR and WKY following cocaine administration by either route.

DISCUSSION

Recently, we have identified RT responses to IV cocaine infusion as a major factor in susceptibility to cocaine-induced toxicity in SHR and WKY rats (6). In the present study, such RT responses to cocaine were examined following different routes of administration and under restrained or freely moving conditions in both SHR and WKY rats. The results demonstrate that route of administration and restraint modify the effects of cocaine on body temperature regulation differentially in these two closely related rat strains.

All freely moving SHR showed significant increases in RT (hyperthermia) following either IV or IP cocaine injection. The hyperthermic response noted under freely moving conditions may result from the well-known, cocaine-induced, increase in muscular activity and locomotion (13). Under conditions of restraint, however, it was evident that SHR demonstrated an heterogeneity of RT response to cocaine challenge. The SHR could be reliably divided into those animals in which the dominant response to cocaine was a hyperthermia (SHR_H) and those in which RT fell (SHR_L). This dichotomy of response was obtained following either IV or IP cocaine injection. Moreover, measurement of an index of the sensitivity to cocaine-induced toxicity, the Tc, indicated that restraint expressed a difference in susceptibility to cocaine toxicity which was related to this subdivision of SHR. With continuous IV cocaine infusion, the time-to-onset of convulsions was significantly shorter in SHR_H than in SHR_L. Therefore, a lower dose of cocaine was required to produce toxicity in the SHR_H when compared to SHR_L. This result confirms our previous data that the susceptibility to cocaine-induced toxicity in SHR is correlated to the degree of increase in RT following cocaine infusion. While this may be considered an artificial subdivision of a highly inbred strain, it is clear from these data, as well as our previous work (6), that a correlation exists between the body

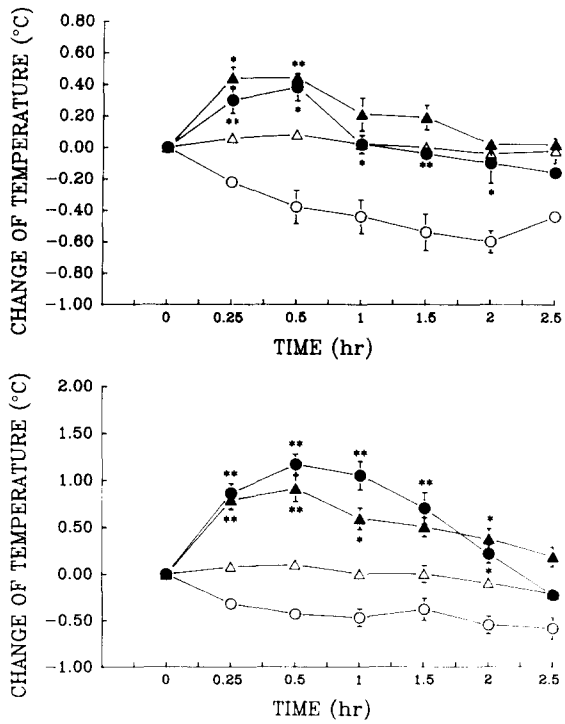

FIG. 3. Rectal temperature responses to IV (upper) and IP (bottom) cocaine in freely moving SHR (circles) and WKY rats (triangles). The filled and open symbols show data obtained from cocaine- and saline-treated animals, respectively. The vertical lines represent the standard errors of the mean. Numbers of animals in each group were, for IV injection: SHR-cocaine, 5; SHR-saline, 6; WKY rats-cocaine, 5; WKY rats-saline, 6 and for IP injection: SHR-cocaine, 16; SHR-saline, 16; WKY rats-cocaine, 10; WKY rats-saline, 10. Asterisks indicate significant differences ($*p<0.05$, $**p<0.01$) compared to corresponding saline-treated groups at corresponding time points.

temperature response to cocaine and the sensitivity to cocaine-induced convulsions in restrained SHR. The designation of SHR into two subgroups is not meant to indicate that two distinct populations of SHR exist, but rather that a variable (i.e., the magnitude and direction of the rectal temperature response to cocaine) exists which can serve as a predictor of the susceptibility to cocaine-induced toxicity.

Hypothermic responses to cocaine in the rat have been reported (4,6) and the hypothesis that physical restraint facilitates heat loss responses and inhibits motor activity in animals has been

TABLE 1

CHANGES IN RECTAL TEMPERATURES IN FREELY MOVING SHR AND WKY RATS

	Intravenous		Intraperitoneal	
SHR	0.74 ± 0.15	(5)	1.60 ± 0.13*	(16)
WKY	0.42 ± 0.07	(5)	0.81 ± 0.17	(10)

Data indicate maximum changes in rectal temperature obtained at 30 min following cocaine injection, relative to saline control groups. $*p<0.01$ compared to all other groups. Results are presented as mean ± S.E.M. The numbers in parentheses indicate the number of animals in each group.

TABLE 2

TIME-TO-ONSET OF CONVULSIONS FOLLOWING COCAINE INFUSION

	SHR_H		SHR_L	
Group A	13.0 ± 0.82	(3)	30.5 ± 3.68*	(3)
Group B	15.3 ± 0.70	(6)	22.8 ± 1.31*[a]	(9)

The SHR of Group A were divided following intravenous injection (1 mg/kg) of a challenge dose of cocaine. Rats in Group B were divided following IP injection (10 mg/kg) of the challenge dose. $*p<0.01$ compared to corresponding SHR_H group. $^a p<0.01$ compared to SHR_L from group A. Results are presented as mean values ± S.E.M. The numbers in parentheses represent the number of animals in each group.

proposed (14). However, it is very interesting that a segment of the SHR population shows hyperthermic responses to cocaine under restrained conditions. The method of restraint employed in the present experiments, i.e., confinement in Plexiglas tubes, might predispose towards heat retention. However, these tubes are open at both ends, save for restraining bars, and have multiple heat dissipation grooves machined along the long axis of each tube. Moreover, hyperthermic responses to cocaine have been demonstrated in dogs (1), rats (2,16) and man (5). A possibility of thermoregulatory dysfunction in SHR has been reported by others (5, 8, 15). Tanaka et al. (15) have reported that thermoregulation in SHR is very labile to exogenous influences such as handling and that the set-point of body temperature is higher than in WKY rats. Thus, while no WKY rats succumbed during a heat (37°C) exposure, 7 out of 13 SHR died (10). Morley et al. (8) used biotelemetry to examine the influence of stress on differential body temperature regulation in SHR and WKY rats. These authors were unable to find differences in resting levels of body temperature between strains, but did demonstrate a marked hyperthermic response to even very mild (handling) stress. Interestingly, a distinct difference in the hyperthermic response of SHR to stress was noted when rats from different commercial breeders were compared. The SHR supplied from Taconic Farms, Inc. (from which animals in the present experiment were obtained also) demonstrated an enhanced hyperthermic response to stress when compared to WKY. A similar difference in responsiveness was not noted in rats obtained from another source. A precise basis for the differential responsiveness of SHR and the heterogeneity of RT responses in SHR which was demonstrated in the present experiments remains to be determined. We have previously noted that neither body weight, nor initial values of rectal temperature, blood pressure and heart rate were different between the two subgroups, SHR_H and SHR_L (6). We have not yet examined the nature of the dose-effect relationship for RT responses in restrained SHR. This experiment and the underlying physiology of the RT response to cocaine in SHR remains to be examined more closely.

Freely moving WKY rats show hyperthermia following either IV or IP cocaine administration. This result also may be explained by secondary effects resulting from cocaine-induced increases in muscular activity. However, unlike SHR, RT responses to cocaine in restrained WKY rats varied with different routes of administration. Intravenous cocaine produced hyperthermia uniformly, while hypothermia was evident in animals in which cocaine was administered by IP injection. These results support previous reports that selected responses to cocaine can vary depending upon the route of drug administration (9,12). Pharmacokinetic variables, including the interval between administration and transport of cocaine to active sites, as well as the local concentrations of cocaine, are likely to vary with different routes of administration (7). A potential influence of behavior, i.e., the presence or ab-

sence of huddling responses in group-housed animals, is unlikely to be a significant factor in the temperature effects. Freely moving rats, whether SHR or WKY, consistently demonstrated hyperthermic responses to cocaine administration, regardless of housing conditions. Animals receiving IV cocaine were, of necessity, housed singly. Animals who received IP cocaine were housed in group cages and did show huddling behavior. All restrained animals were housed individually during cocaine administration, regardless of the route of administration. The reversal of RT responses induced by different routes of administration was evident under only restraint. Thus, restraint stress appears to play an important role in the reversal of RT responses to cocaine following different routes of administration in WKY rats. The results in WKY rats also support the hypothesis that restraint facilitates heat loss responses, in part by inhibiting motor activity.

The relationship between restraint and the response of RT to drug administration is not unique to cocaine. Spencer et al. (14), using opiate receptor agonists, have reported results similar to those observed with cocaine. In their studies, administration of a mu-opioid receptor agonist was shown to decrease body temperature in restrained rats, while hyperthermia was produced in unrestrained rats. A recent publication has emphasized the differential thermoregulatory responses of SHR and WKY to restraint itself (8).

Factors other than restraint stress and route of administration further modify RT responses to cocaine. The magnitude of RT responses to cocaine in the freely moving state was relatively larger in SHR than in WKY rats, a fact that was particularly evident when comparisons were made between responses following cocaine and those from corresponding saline-treated groups. Injection of saline vehicle in SHR reduced RT by both IV and IP routes. In contrast, no changes in RT were observed in WKY rats following administration of saline. It is unclear, at present, whether this response reflects alterations in heat loss or in heat production. Others have reported that SHR are more sensitive to apomorphine-induced hypothermia than are WKY rats (4).

In conclusion, restraint significantly modifies the responses of body temperature to cocaine in both SHR and WKY rats. The possibility that the arbitrary grouping of restrained SHR into the subgroups of SHR_H and SHR_L reflects the existence of two distinct populations is an area for further investigation. Additionally, although an importance of route of administration in thermoregulatory responses to cocaine was demonstrated in restrained WKY rats, further studies concerning dose-response relationships must be performed.

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