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BRIEF COMMUNICATION

Delta Sleep-Inducing Peptide in Spontaneously Hypertensive Rats

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GRAF, M. V., A. J. KASTIN AND G. A. SCHOENENBERGER. *Delta sleep-inducing peptide in spontaneously hypertensive rats.* PHARMACOL BIOCHEM BEHAV 24(6) 1797–1799, 1986.—Delta sleep-inducing peptide has been shown to exert extra-sleep effects as well as effects on sleep. In this study, the concentrations of DSIP-like immunoreactivity were measured by radioimmunoassay in the plasma of spontaneously hypertensive rats (SHR). They were found to be about 25% higher in SHR plasma than in the plasma of the normotensive Wistar-Kyoto (WK) controls. DSIP was then infused for 10 days by osmotic minipump (200 μg/kg/day) into SHR. This resulted in maintenance of BP at a level of about 200 mm Hg as compared with the significant increase to about 220 mm Hg after 10 days in the SHR controls infused with 0.9% NaCl. After daily SC injection of a single dose of 200 μg/kg DSIP for each of 5 days in SHR, findings were similar. The results raise the possibility of an involvement of DSIP in the regulation of BP in SHR.

Delta-sleep-inducing peptide Hypertension Rat Stress

THE isolation and characterization of delta-sleep-inducing peptide (DSIP) was reported almost 10 years ago [17,18]. Since then, the synthetic peptide has been used in many investigations demonstrating sleep as well as extra-sleep activities of DSIP [3,9]. The chance observation of an apparent improvement in the hypertension of a patient receiving DSIP for a different purpose suggested that the possible interaction of DSIP and blood pressure (BP) be examined in the laboratory.

METHOD

DSIP was a generous gift from Dr. D. Gillessen, F. Hoffmann-La Roche, Basel, Switzerland. Alzet osmotic minipumps, model 2001, were obtained from Alza Corporation, Palo Alto, CA.

Male SHR and WK rats (6 weeks old) used for determination of DSIP-like immunoreactivity (DSIP-LI) in plasma were purchased from Charles River Breeding Laboratories, Wilmington, MA. Male SHR (10 weeks old) used for studies of BP were obtained from Madorin AG, Fullinsdorf, Switzerland. The animals were kept for at least 5 days under a 12 hr light:dark regimen with free access to food and water. Determination of DSIP-LI in plasma was performed as described elsewhere [5] with antibody No. 607.

Measurement of BP at 0900 hr the first day in both experiments served as baseline. DSIP was administered subcutaneously (SC) by two different techniques. In one method, single injections of DSIP (200/μg/kg) or 0.9% NaCl (controls) were given for 5 consecutive days between 0900 hr and 1000 hr. One hour after injection, systolic BP was measured and recorded with a W+W BP recorder 8008 (W+W Electronic AG, Basel, Switzerland) by the indirect tail-cuff method on prewarmed animals. Each score was the mean of at least 2 measurements.

In another experiment, 2 groups of SHR were implanted with Alzet minipumps beneath the skin of the back under light ether anesthesia. The pumps of one group were filled with DSIP in 0.9% NaCl whereas the control group contained only the physiological saline. According to the manufacturer, the pumps released 21–22 μl of solution per day corresponding to 200 μg/kg DSIP in the test group. BP was measured 1, 3, 7, and 10 days after implantation of the pumps.

On the 10th day, the rats were exposed to ether for 30 sec and killed by decapitation 25 min later. Trunk blood was collected into chilled tubes containing EDTA. Corticosterone in plasma was determined according to Ruch *et al.* [11]. Blood chemistries were analyzed in plasma by the routine methods of the central laboratory of the hospital in Basel.

Evaluation of the results was performed when appropriate by analysis of covariance (ANOCOVA). This was followed by Duncan's Multiple Range Test.

RESULTS

The mean (±SEM) concentration of DSIP-LI in the

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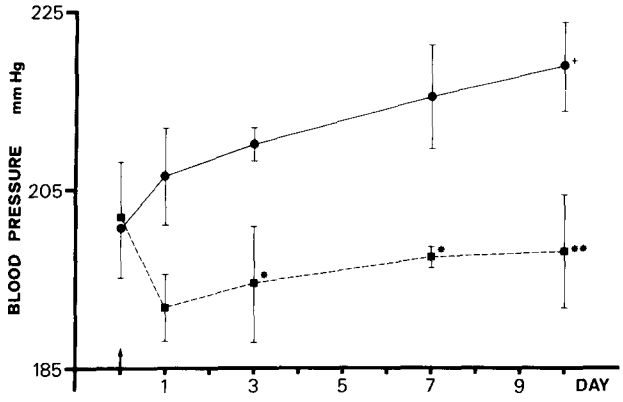

FIG. 1. Effect of chronic infusion of DSIP (dotted line) on BP in SHR. The values are means±SEM of 5 rats. Baseline values (arrow) were measured at day 0 before implantation of the minipumps. $^{*}p<0.05$, $^{**}p<0.01$ compared with the corresponding control; $^{+}p<0.05$ compared with baseline values.

plasma of 11 SHR was 509±34 pg/ml whereas in the 10 WK controls, the mean concentration was 401±20 pg/ml. Comparison of the two means by a t-test revealed that the difference in the concentration of DSIP-LI in the plasma of the groups was statistically significant ($p<0.05$).

Six rats in each of 2 groups were injected daily SC with either 200 μg/kg DSIP or 0.9% NaCl. BP was measured before the first injection and 1 hr afterwards. ANOCOVA with the baseline level as the covariate revealed a significant influence of time, $F(4,40)=12.34$; $p<0.001$, reflected by the significantly ($p<0.01$) increased mean BP in controls after 5 days (210.3±4.4 mm Hg) as compared with baseline (192.5±3.1 mm Hg). The main effect of treatment did not reach significance, $F(1,40)=3.96$; $p<0.08$, but the interaction of time by treatment was significant, $F(4,40)=2.86$; $p<0.05$. The mean BP of DSIP-treated rats was significantly lower than that of the saline controls on days 1, 4, and 5, but not on days 2 or 3. On day 4, the mean score of the group receiving DSIP (183.5±3.2 mm Hg) was significantly ($p<0.05$) lower than baseline (193.3±4.0 mm Hg).

BP also was measured in 10 SHR before implantation of osmotic pumps containing DSIP (n=5) or saline (n=5). During the following 10 days, BP was determined on days 1, 3, 7, and 10. The results are shown in Fig. 1. Baseline pressures in the 2 groups were not different. The influence of continuous DSIP infusion was highly significant, $F(1,24)=17.22$; $p<0.005$, whereas neither the time nor the interaction of treatment by time revealed any significant effect. Comparison of the paired means showed reduced pressures in DSIP-treated rats compared with controls on days 3, 7, and 10 after implantation. The higher mean pressure in the control group on day 10 was the only value significantly higher ($p<0.05$) than baseline.

In the rats implanted with minipumps, the possible effect of body weight was also determined. There was a clear effect of baseline weight, $F(1,16)=133.4$; $p<0.001$, and a significant effect of time on weight gain, $F(2,16)=76.2$; $p<0.001$, but no difference due to treatment or interaction of the peptide with time was found. No differences in sleep were observed.

Twenty-five minutes after exposure to ether on the 10th day after implantation of the minipumps, the concentration of corticosterone in plasma tended to be increased. The mean concentration in SHR receiving saline was 22.6±3.8 μg% as compared with 14.7±0.9 μg% in SHR receiving DSIP ($p<0.1$).

Almost all chemistries (sodium, urea, inorganic phosphate, iron, chloride, glucose, total bilirubin, aspartate aminotransferase, alanine aminotransferase, cholesterol, triglycerides, total protein, creatine kinase, alpha-amylase, lactate dehydrogenase) measured in the plasma of these rats showed similar means. However, the percent of the total protein for albumin was lower (46.5±2.1% after DSIP vs. 51.6±1.6% after saline; $p<0.01$) and that for alpha-1-globulin (17.8±1.0% vs. 15.5±0.4%; $p<0.01$) and gamma-globulin (5.2±1.2% vs. 2.8±0.3%; $p<0.01$) higher in DSIP-treated rats.

DISCUSSION

Since an increasing effect of DSIP with repeated injections has been observed in several investigations [1, 8, 14–16], DSIP was administered in the present studies either by single daily injections for 5 days or by continuous infusion with a minipump for 10 days. DSIP appeared to result in a stabilization of BP at the basel level whereas BP in the controls steadily increased over time. Yet, levels of DSIP-LI in SHR were higher than in the normotensive WK rats even though infusion of DSIP in SHR resulted in lower BP. Although DSIP may interact with the adrenergic system in some situations [6,7], it is not known whether the effects of DSIP on BP in SHR were mediated through modulation of adrenergic transmission.

DSIP has been reported to increase tolerance against stress [3, 10, 12, 13, 15]. The exposure to ether of SHR implanted with minipumps for 10 days tended to result in relatively lower concentrations of corticosterone in the rats receiving DSIP. Attenuation by DSIP of the increase in corticosterone levels induced by corticotropin-releasing factor (CRF) was found in a different study [4], and reduced concentrations of corticosterone have been observed in the evening 4 hr after injection of DSIP [2]. It cannot be excluded that the effects of DSIP on BP were mediated by an interaction with the regulation of corticosterone since the adrenal cortex may play an important role in the development of hypertension in SHR [11]. It also is not known whether the exposure to ether could have affected the plasma proteins, but complex changes in these substances were found in an earlier study after injection of DSIP [2].

Thus, the results of the present study show an effect of DSIP on BP in SHR. They also show altered concentrations of DSIP-LI in the plasma of SHR.

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