observed when either 1 µg A II (n = 5) or the solvent alone (n = 3; not shown) were used. I.c.v. injections of A II also increased blood pressure in a dose-dependent fashion. Results were similar in the 2 animals studied and are presented in figure 2 for monkey 112, in which blood pressure increments of 6, 19 and 46 mm Hg were observed after administration of 1, 10 and 50 µg A II respectively.

The effect of A II on PRL release in the monkey differs from that described in the rat. In the latter, Steele et al.[15] have found that i.c.v.-injections of A II lowered PRL plasma levels. Such a discrepancy may be attributed to species differences in the distribution of A II binding sites[16]. The present study indicates that A II may be involved in the control of PRL secretion in primates. The observation that i.c.v.-injections of A II modify in a parallel way both blood pressure and PRL plasma levels suggests the possibility of a link between the 2 phenomena. However, our results do not allow us to decide whether A II increases PRL secretion by acting directly on the nervous mechanisms involved in hormone release or indirectly, via its central effect on arterial blood pressure.

1 Acknowledgments. We thank Mrs J. Arsaut and D. Verrier for excellent technical assistance. This work was supported by grants from INSERM (CRL No. 78.1.2656), DGRST (77.7.9654) and CNRS (ERA 493).
2 J. Meites and C.S. Nicoll, Rev. Physiol. *28*, 57 (1966).
3 A.V. Schally, T.W. Redding, R.A. Arimura, A. Dupont and G.L. Linthicum, Endocrinology *100*, 681 (1977).
4 G. Racagni, J.A. Apud, V. Locatelli, D. Cocchi, G. Nistico, R.M. Di Giorgio and E.E. Muller, Nature *281*, 575 (1979).
5 A. Dupont, L. Cusan, F. Labrie, D.H. Cox and C.J. Li, Biochem. biophys. Res. Commun. *75*, 76 (1977).
6 C. Rivier, M. Brown and W. Vale, Endocrinology *100*, 751 (1977).
7 M. Ruberg, W.H. Rotsztejn, S. Arancibia, J. Besson and A. Enjalbert, Eur. J. Pharmac. *51*, 319 (1978).
8 J.T. Fitzsimons, Physiol. Rev. *52*, 468 (1972).
9 G. Simonnet, F. Rodriguez, F. Fumoux, P. Czernichow and J.D. Vincent, Am. J. Physiol. *237*, R 20 (1979).
10 D.G. Changaris, L.C. Keil and W.B. Severs, Neuroendocrinology *25*, 257 (1978).
11 J.T. Quinlan and I. Philips, Brain Res. *205*, 212 (1981).
12 W.B. Severs, J. Summy-Long, J.S. Taylor and J.D. Connor, J. Pharmac. exp. Ther. *174*, 27 (1970).
13 A.M. Reuter, F. Kennes, Y. Gevaert and P. Franchimont, Int. J. nucl. Med. Biol. *3*, 21 (1976).
14 WP-XI-49-29 (biological activity 40 IU/mg) was a gift of Dr W.D. Peckham (Pittsburgh, USA).
15 M.K. Steele, A. Negro-Vilar and S.M. McCann, Endocrinology *109*, 893 (1981).
16 J.W. Hardling, L.P. Stone and J.W. Wright, Brain Res. *205*, 265 (1981).

Hypothyroidism lowers blood pressure, adenylate cyclase and Na^+,K^+- and K^+,Ca^{++}-ATPase activities in normotensive and spontaneously hypertensive rats[1]

S.J. Blumenthal, M.M. McConnaughey and S.G. Iams

Departments of Physiology and Pharmacology, East Carolina University, School of Medicine, Greenville (North Carolina 27834, USA), 25 February 1981

Summary. Myocardial isoproterenol-stimulated adenylate cyclase, Na^+,K^+-ATPase and K^+,Ca^{++}-ATPase activities are elevated in the spontaneously hypertensive rat and can be lowered by methimazole-induced hypothyroidism which also prevents the development of hypertension. Although thyroid hormone levels are similar between untreated SHRs and WKY rats, the thyroid is apparently necessary for the expression of spontaneous hypertension.

The spontaneously hypertensive rat (SHR) displays a transitory increase in cardiac index during the development of hypertension[2] similar to that observed in some forms of human hypertension[3]. The altered reactivity of the SHR myocardium may be due to several factors including thyroid status and the activities of adenylate cyclase or the electrolyte transporting enzymes Na^+,K^+-ATPase and K^+,Ca^{++}-ATPase.

Since hypothyroidism attenuates hypertension in the SHR[4] and lowers adenylate cyclase activity[5], an alteration in this enzyme may be a factor in the development or maintenance of hypertension. Hypothyroidism has been shown to alter myocardial Na^+,K^+-ATPase as well as K^+,Ca^{++}-ATPase activity[6] and alterations in these ion transporting enzymes could be one possible explanation for alterations in the contractile properties and reactivity of the SHR myocardium.

The present study was undertaken to determine if, during the development of hypertension, changes occur in adenylate cyclase, ATPase enzymes, or thyroid hormone levels which could account for the altered cardiac index observed in the prehypertensive SHR. Since these enzyme activities of the myocardium appear to be altered by thyroid hormones they were also assessed in hypothyroid animals.

Methods. Hypothyroidism was induced in neonate SHR and WKY rats by the continuous administration of 0.01% methimazole via the drinking water to lactating dames and later to the weaned rats. Blood pressure was measured with a Friedman: Freed Microphonic manometer and tail cuff on conscious, restrained rats after gentle prewarming. Rats were sacrificed at 0, 5, 10, 20, 30 and 100 days of age by decapitation. The blood serum of each group was collected, pooled and stored below -25 °C. The ventricles were rapidly removed and placed in ice-cold homogenization buffer (250 mM sucrose/50 mM Tris HCl, pH 7.5). The pooled ventricles of each group were homogenized in ice-cold homogenization buffer (10 ml/g) by 3 30-sec pulses of a Brinkmann Polytron PT-10 tissue homogenizer ($^1/_2$ maximal speed). The homogenate was centrifuged at 4 °C, 1000 × g for 10 min. The supernatant was centrifuged at 4 °C at 40000 × g for 30 min and the resulting pellet was resuspended in homogenization buffer (approximately 8 mg protein/ml). Protein was determined by the method of Lowry et al.[7]. Adenylate cyclase activity was assessed by measuring the conversion of [$\alpha - ^{32}$P] ATP to cyclic [^{32}P] AMP[8]. ATPase activities were measured at 37 °C by monitoring the release of inorganic phosphorus from 3 mM Tris ATP[9]. Total Na^+,K^+-ATPase activity[10] and K^+,Ca^{++}-ATPase activity[11] were measured by previously determined protocols. Serum T_3 and T_4 levels were measured by competitive binding RIA using procedures outlined by Clinical Assay Division of Travenol Laboratories. Statistical significance was by Student's t-test and analysis of covariance with age as the covariant.

Results. Serum T_3 levels in control neonates were below the range of the assay until the rats of both strains reached 10 days of age. The maximum levels for both SHRs and WKY rats were reached by 30 days of age (1.50 and 1.22 ng/ml respectively) and dropped slightly by 100 days. Serum free T_4 levels for SHRs and WKYs reached adult levels by 20 days (0.92 and 1.08 ng/ml) and decreased thereafter until last measured at 100 days (0.47 and 0.36 ng/ml). Serum total T_4 levels for SHRs continued to rise throughout the experiment (100 days: 5.59 µg/100 ml). WKY rats reached their maximum levels of total T_4 at 20 days (4.20 µg/100/ml) and decreased slightly thereafter. Serum T_3, free T_4 and total T_4 levels were similar in both strains of rats. Methimazole treatment significantly lowered ($p < 0.05$) total T_3 in the SHR; serum T_3 levels in methimazole-treated SHRs were below the range of the assay at all ages except 20 days. Measurable levels below controls were observed for most ages after 10 days in the WKY. Methimazole significantly lowered ($p < 0.05$) free T_4 and total T_4 in both strains. Blood pressures (mm Hg) in the control SHRs vs control WKY rats were significantly higher ($p < 0.05$) at all ages measured (30 days: 123 ± 2 vs 103 ± 4; 100 days: 192 ± 3 vs 134 ± 4). Methimazole treatment significantly lowered ($p < 0.05$) blood pressures in the SHRs and WKY rats at all ages (30 days: 99 ± 2 and 80 ± 5; 100 days: 122 ± 2 and 106 ± 4). Hypothyroid SHRs exhibited blood pressures similar to or lower than those associated with normotensive control WKY rats. Basal cardiac adenylate cyclase activities (pmoles cAMP/mg protein/min) were slightly higher in newborn SHRs when compared to WKYs and decreased with age to about the same levels in both the SHRs and WKY rats (0 day: 67 ± 12 and 47 ± 1; 100 days: 17 ± 1 and 23 ± 2). Guanine nucleotide-stimulated adenylate cyclase activities were also slightly higher in the newborn SHRs when compared to WKYs and also decreased with age to similar levels in the SHRs and WKY rats (0 days: 80 ± 14 and 60 ± 14; 100 days: 39 ± 0 and 42 ± 1). Dose response curves to the stimulation of adenylate cyclase activities by GTP showed a similar value for half maximal stimulation in membranes from control and hypothyroid SHRs and WKY rats. In all cases the GTP concentration required for half maximal cyclase stimulation fell between 8×10^{-7} and 9×10^{-7}M. Isoproterenol-stimulated adenylate cyclase activities (1 µM isoproterenol) assessed in cardiac membranes from SHRs were significantly higher ($p < 0.05$) than those activities assessed from WKY rats at early ages (0–30 days) (fig. 1). This increase was reversed by 100 days of age where the hormone-stimulated activity in the SHRs was 16% lower ($p < 0.05$) than in the WKY rats. Methimazole treatment significantly lowered ($p < 0.05$) all cardiac adenylate cyclase activities tested in membranes from SHRs to values similar to those assessed in normotensive control WKY rats through 30 days of age (fig. 1). Although adenylate cyclase activities were also attenuated by methimazole treatment in the WKY rats, these decreases were not significant. As shown in figure 2 isoproterenol dose response curves indicate the half maximal stimulation to be similar in both control and methimazole treated rats.

The activity of the Na^+, K^+-ATPase (µmoles Pi/mg protein/h) was significantly higher ($p < 0.05$) in cardiac membranes prepared from SHRs when compared to WKY rats at all ages except 0–5 days (100 days: 6.2 ± 0.6 and 5.1 ± 0.2). The activities of this enzyme did not decrease

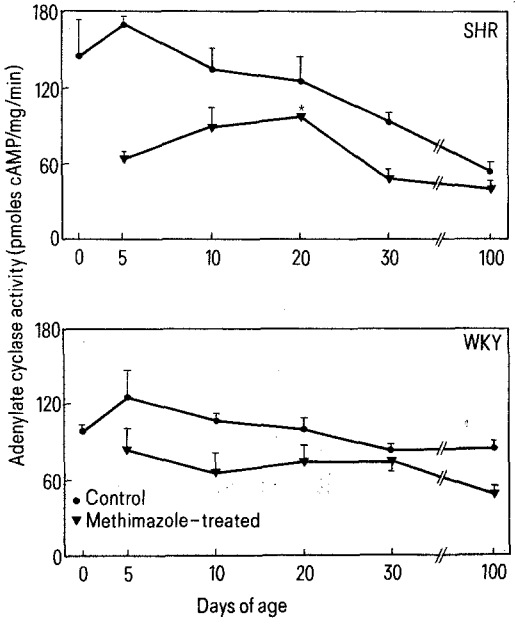

Figure 1. Effect of methimazole on isoproterenol-stimulated adenylate cyclase activities in cardiac membranes of SHRs and WKY rats. Cardiac membrane protein (40 µg) was incubated with 1 µM 5′-guanylimidophosphate and 1 µM isoproterenol as described in 'methods'. Methimazole treatment significantly lowered ($p < 0.05$) the isoproterenol-stimulated activities in SHR myocardial preparations through 30 days of age. Each point represents the mean ± SE of 2–8 pooled preparations. * 1 determination only.

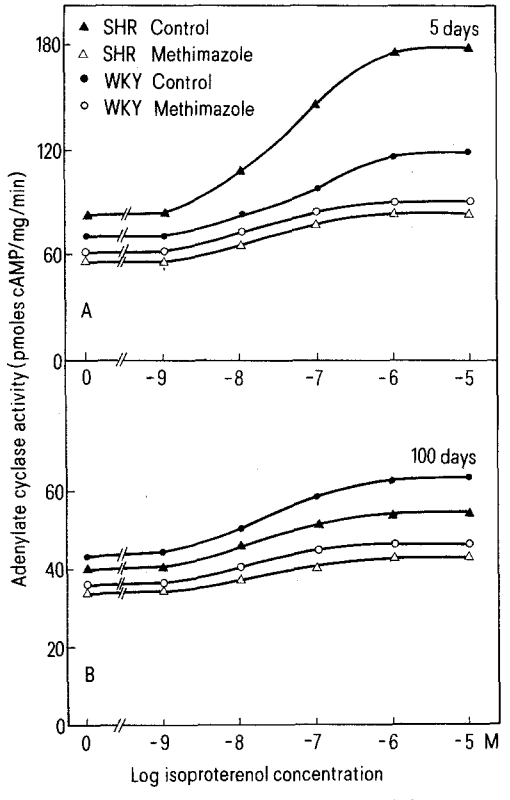

Figure 2. Effect of methimazole on isoproterenol dose response for 2 ages of rats in cardiac membranes of SHRs and WKY rats. Cardiac membrane protein (40 µg) was incubated with 1 µM 5′-guanylimidophosphate and varying concentrations of isoproterenol. Methimazole treatment had no effect on the half maximal stimulation by isoproterenol in either strain. Panel A represents animals 5 days of age and panel B represents animals of 100 days of age. Each value represents the mean of triplicate incubations. Standard errors were not included as they were all less than 3%.

with age as was seen with adenylate cyclase activities. Na^+,K^+-ATPase activities were significantly decreased ($p < 0.05$) in cardiac preparations from both strains of rats treated with methimazole (100 days: SHR 3.4 ± 0.4; WKY 2.4 ± 0.3). The activity of the K^+,Ca^{++}-ATPase (μmoles Pi/mg protein/h) was significantly higher ($p < 0.05$) in cardiac membranes prepared from SHRs when compared to activities from membranes of WKY rats at all ages tested (100 days: 6.5 ± 0.4 and 5.5 ± 0.5). This ATPase activity of the sarcoplasmic reticulum also did not decrease with age as was seen with adenylate cyclase activities. Methimazole treatment decreased K^+,Ca^{++}-ATPase activities ($p < 0.05$) in both strains of rats at all ages (100 days: SHR 4.0 ± 0.3; WKY 3.1 ± 0.5).

Discussion. The results of this study suggest that no differences exist in thyroid hormone levels between SHRs and WKY rats. Methimazole treatment produced hypothyroidism and lowered blood pressure in the SHRs to values similar to those observed in the untreated normotensive WKY rats. The increased cardiac index observed in the early stage in the development of hypertension[2] may be indicative of an increased beta-adrenergic reactivity. Prehypertensive SHRs and age matched WKY rats exhibit similar apparent numbers of myocardial beta-adrenergic receptors and methimazole treatment does not significantly alter the numbers or apparent K_D for this receptor[12]. The elevated isoproterenol-stimulated adenylate cyclase activity observed in the prehypertensive SHR may contribute to the elvated cardiac index seen in the development of hypertension. We confirm previous reports that hypothyroidism will lower this enzymatic activity[5]. The catecholamine-stimulated adenylate cyclase activity of the SHR can be decreased to or below the levels observed in the normotensive WKY rats by methimazole treatment up to 100 days of age. This lower activity may be in part responsible for the lower blood pressures seen in the treated SHR.

We confirm previous reports of elevated Na^+,K^+-ATPase (13) and K^+,Ca^{++}-ATPase[14] activities for the SHR with established hypertension and have demonstrated that these activities are also elevated in the prehypertensive SHR. These elevated enzyme activities may reflect responses secondary to alterations in membrane permeability to various electrolytes. The elevated adenylate cyclase activities which decrease as hypertension appears may be intimately involved in the development of hypertension in the SHR. Methimazole-induced hypothyroidism significantly decreased all activities and blood pressure thus suggesting the thyroid to be necessary for the complete expression of hypertension.

1 Acknowledgments. Supported by North Carolina Heart Grant No. 40301 and a grant from Sigma Xi.
2 T.L. Smith and P.M. Hutchins, Hypertension *1*, 508 (1979).
3 E.D. Frohlich, V.J. Kozul, R.C. Tarazi and H.P. Dustan, Circulation Res. *26*, suppl. 1, 27 (1970).
4 S.G. Iams and S.J. Blumenthal, Physiologist *22*, 60 (1979).
5 R.V. Sharma, O. Habhab and R.C. Bhalla, Biochem. Pharmac. *28*, 2858 (1979).
6 M.M. McConnaughey, L.R. Jones, A.M. Watanabe, H.R. Besch, Jr, L.T. Williams and R.J. Lefkowitz, J. cardiovasc. Pharmac. *1*, 609 (1979).
7 O.H. Lowry, N.J. Roseborough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
8 L.R. Jones, H.R. Besch, Jr, J.W. Fleming, M.M. McConnaughey and A.M. Watanabe, J. biol. Chem. *254*, 230 (1979).
9 L.R. Jones, H.R. Besch, Jr, and A.M. Watanabe, J. biol. Chem. *253*, 1643 (1978).
10 H.R. Besch, Jr, L.R. Jones and A.M. Watanabe, Circulation Res. *39*, 586 (1976).
11 L.R. Jones, H.R. Besch, Jr, and A.M. Watanabe, J. biol. Chem. *252*, 3315 (1977).
12 S.J. Blumenthal, M.M. McConnaughey and S.G. Iams, Endocrinology *106*, suppl., 169 (1980).
13 T. Godfraind and F. Noel, Archs int. Pharmacodyn. *245*, 139 (1980).
14 K. Aoki, N. Ikeda, K. Yamashita, K. Taxumi, I. Sato and K. Hotta, Jap. Circul. J. *38*, 1115 (1974).

CONGRESSUS

Austria

13th international congress of chemotherapy

Vienna, 28 August–2 September 1983

The scientific program of this congress to be held at the Kongresszentrum-Hofburg in Vienna will consist of main lectures, symposia, round table sessions and free papers with posters, and will include antimicrobial as well as anticancer topics. Further information, also concerning deadlines, etc., through the Secretariat of the 13th Int. Congress of Chemotherapy, or through Prof. K. Karrer, Institute for Cancer Research, University of Vienna, Borschkegasse 8a, A-1090 Vienna/Austria.

Austria

9th international mass spectrometry conference

Vienna, 30 August–3 September 1982

The conference is organized by the Austrian Mass Spectrometry Group, the Austrian Society for Microchemistry and Analytical Chemistry, the Austrian Chemical Society and the Institute of Analytical Chemistry of the University of Vienna in cooperation with an international scientific committee.

The program covers invited lectures, submitted papers and posters. For information please contact the secretariat of the conference: Interconvention, P.O. Box 105, A-1014 Vienna/Austria.