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Temperature Sensitivity of Angiotensin-Induced Inotropic Effects (41910)

GEORGE J. TRACHTE AND MICHAEL J. PEACH*

Department of Pharmacology, School of Medicine, University of Minnesota, Duluth, Minnesota 55812, and
**Department of Pharmacology, School of Medicine, University of Virginia, Charlottesville, Virginia 22908*

Abstract. Temperatures in the range of 30 to 37°C were examined for effects on angiotensin (Ang) peptide-induced inotropic actions in rabbit isolated aortic rings and left atria. Both transmurally-stimulated and punctate-stimulated atria were employed to examine neurogenic and direct cardiac actions of the Ang, respectively. The peptides examined were Ang II, Ang III, and sarcosyl[1]-Ang II. Ang III was more potent at 33°C than at 37°C in the transmurally stimulated atria, in which the Ang III concentration–response curve was shifted roughly 10-fold to the left at 33°C in comparison to 37°C. Sarcosyl[1]-Ang II responses were maximal at 37°C and significantly reduced at lower temperatures in aortic rings (30°C) and in transmurally stimulated atria (30 and 33°C). Ang II actions were not significantly altered by temperature changes in the 30 to 37°C range except in transmurally stimulated atria, in which angiotensin II was more potent at 30°C than at 33°C. The results indicate that relatively small temperature alterations can profoundly affect potency ratios and maximal responses among the angiotensin peptides particularly when the angiotensin response is mediated in part by activation of sympathetic neurons, as in the transmurally stimulated atria. The data are consistent with either different mechanisms of cellular stimulation or multiple populations of receptors for the angiotensins. The greater effect of temperature alterations on angiotensin responses in the transmurally stimulated atria suggests that the peptides influence sympathetic nerves and cardiac or smooth muscle differently.

Angiotensins II and III induce an increase in the force of contraction of isolated cardiac preparations by a receptor-mediated action on calcium channels (1, 2). However, angiotensins also enhance norepinephrine release from myocardial adrenergic nerve endings (3, 4) and reduce norepinephrine uptake by cardiac tissue (5). In the transmurally-stimulated isolated rabbit left atrium, angiotensin II enhances the release of dopamine-β-hydroxylase (6) and potentiates contractile responses to the indirectly acting sympathomimetic, tyramine (7). These data indicate that a component of the angiotensin-induced inotropic effect in the transmurally-stimulated atria is mediated via an action on sympathetic nerve terminals to enhance the release of neurotransmitters. In contrast, punctate-stimulated atrial responses to angiotensin II are devoid of an adrenergic component (6).

We observed a large difference in the potencies of angiotensins II and III in transmurally-stimulated atria at 37°C. Previous data from this laboratory indicated a much smaller difference in potencies in transmurally-stimulated atria at 30°C (7). The present study was designed to test the hypothesis that the potency and/or efficacy of angiotensin peptides are dependent upon temperature. Sarcosyl[1]-angiotensin II was included in the study because it is resistant to metabolism by aminopeptidase A (8). Therefore, sarcosyl[1]-angiotensin II is less likely to be converted to the heptapeptide, angiotensin III, and may be a better representative of an angiotensin octapeptide than angiotensin II, which can undergo conversion to angiotensin III. The actions of angiotensins II and III and sarcosyl[1]-angiotensin II were examined in two types of atrial preparations, punctate-stimulated and transmurally-stimulated, to examine direct cardiac effects and a combination of cardiac and neurogenic effects, respectively. These peptides were also examined in a vascular preparation, the isolated aortic ring, to determine whether isolated vascular smooth muscle responsiveness was dependent on temperature. The results may be relevant as an explanation for the varying potency ratios reported for angiotensin peptides. Temperature sensitivities of catecholamines have been explained by receptor conversion from β to α (9). Therefore, data on temperature sensitivities of the angiotensins may be

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of value in establishing either the mechanisms of cellular activation or the presence of separate populations of receptors for the angiotensin peptides.

Materials and Methods. Male New Zealand white rabbits were killed by cervical dislocation and hearts and aortae were removed and placed in oxygenated Krebs–bicarbonate solution. The Krebs–bicarbonate buffer contained the following: 112 m*M* sodium chloride, 25 m*M* sodium bicarbonate, 4.5 m*M* potassium chloride, 0.5 m*M* magnesium chloride, 1.2 m*M* sodium phosphate monobasic, 11 m*M* glucose, and 2.5 m*M* calcium chloride. The left atria were removed from the hearts and split into two halves. Each half was attached to a Grass Ft03C force transducer and placed in a water-jacketed, 10-ml chamber containing Krebs–bicarbonate buffer gassed with 95% O_2:5% CO_2. The resting tension was set at 4.5*g* which Blumberg *et al.* (7) found to result in maximal force generation in response to an electrical stimulus. We also confirmed that this tension resulted in a maximum contraction on the length–tension curve. One atrial preparation was stimulated transmurally at twice threshold with platinum electrodes. Threshold voltage for contraction averaged 21 ± 3 V. The other atrial half was utilized as a punctate-stimulated preparation. The atria were anchored on one electrode, and the other electrode was inserted into a longitudinal slit in the atria. In this way both electrodes contacted the tissue directly. The free end of the atria was attached to a force transducer with silk suture to measure isometric force generation. Threshold voltage averaged 2.5 ± 0.2 V and the preparations were stimulated at twice threshold voltage. Electrical stimuli were 10 msec in duration at a frequency of 1 Hz. Aortic rings, approximately 3 mm wide, were cut from the thoracic aorta. Rings were attached to a horizontal support and connected to Grass Ft03C force transducers with a square wire hook. A resting tension of 1.5 g resulted in maximal contractile responses to constrictor agents. Results are reported as increases in isometric force generated in response to the angiotensin peptides. All contractile results were recorded on a Grass Model 7D polygraph.

Temperature in the chambers was regulated by a Haake FE2 circulating water bath. All preparations were allowed to equilibrate 2 hr before being exposed to any peptides. The preparations were subsequently allowed to reequilibrate for at least 60 min after an alteration in temperature. Cumulative angiotensin concentration–response curves were obtained at 30, 33, and 37°C. Only two concentration response curves were performed per preparation per day. The upper limit of angiotensin concentrations employed was set at 10^{-5} *M* because surfactant activity of the angiotensins limited their concentrations in the bathing fluid above this concentration.

Angiotensins II and III were obtained from U.S. Biochemical Corporation (Cleveland, Ohio). Purity of these peptides was confirmed by the presence of only one fluorescamine-reactive spot on silica gel thin-layer chromatography plates after development in the solvent system of butanol:acetic acid:pyridine:water (15:3:10:12). Sarcosyl[1]-angiotensin II was a gift from Dr. A. Chiu (Oklahoma City, Okla.).

Potencies were estimated by fitting each curve to a logarithmic plot with a standard

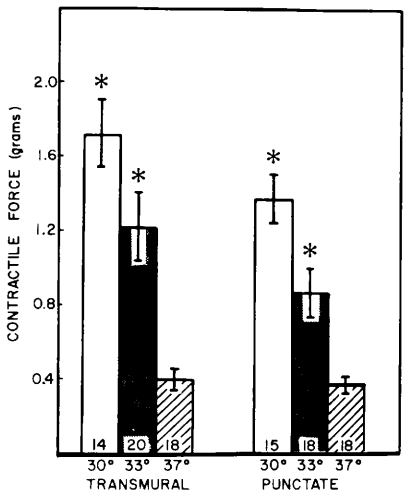

FIG. 1. The effect of temperature on the control force generation of isolated transmurally stimulated and punctate-stimulated atria. Columns represent the mean contraction for a given temperature, numbers within the columns represent the number of donor animals per group, and bars are the SEM. * $P < 0.001$ in comparison to 37°C.

Hewlitt–Packard 67 program determining the EC_{50}. Comparisons of both the EC_{50} and contractile responses were made by the Student t test. A P value less than 0.05 was considered significant.

Results. The effect of temperature on basal contractions of transmurally-stimulated and punctate-stimulated atria at different temperatures is shown in Fig. 1. Contractility was decreased by increasing the temperature from 30 to 37°C. The contractile force at 37°C was significantly different from that at 30 or 33°C ($P < 0.001$). Contractile responses to punctate and transmural stimulation were not significantly different at any temperature. These results demonstrate that cardiac contractions are enhanced two- to three-fold by a temperature reduction of 4°C from the physiological temperature of 37°C.

The effect of temperature on the angiotensin concentration response curves in transmurally-stimulated atria is demonstrated in Fig. 2. At 30°C all peptides exhibited similar maximal responses, as shown in Table I. The order of potency, as defined by the EC_{50}, was sarcosyl[1]-angiotensin II > angiotensin II > angiotensin III, in the ratio of 26:20:1. These data are presented in Table II. Increasing the temperature to 33°C enhanced the potency of angiotensin III and reduced maximal responses to sarcosyl[1]-angiotensin II. Maximal effects of sarcosyl[1]-angiotensin II at 33°C were significantly suppressed relative to those of angiotensins II or III ($P < 0.05$) and to maximal responses of sarcosyl[1]-angiotensin II at 30 or 37°C ($P < 0.01$). Maximal inotropic responses to angiotensin II at 33°C were not significantly different from those at

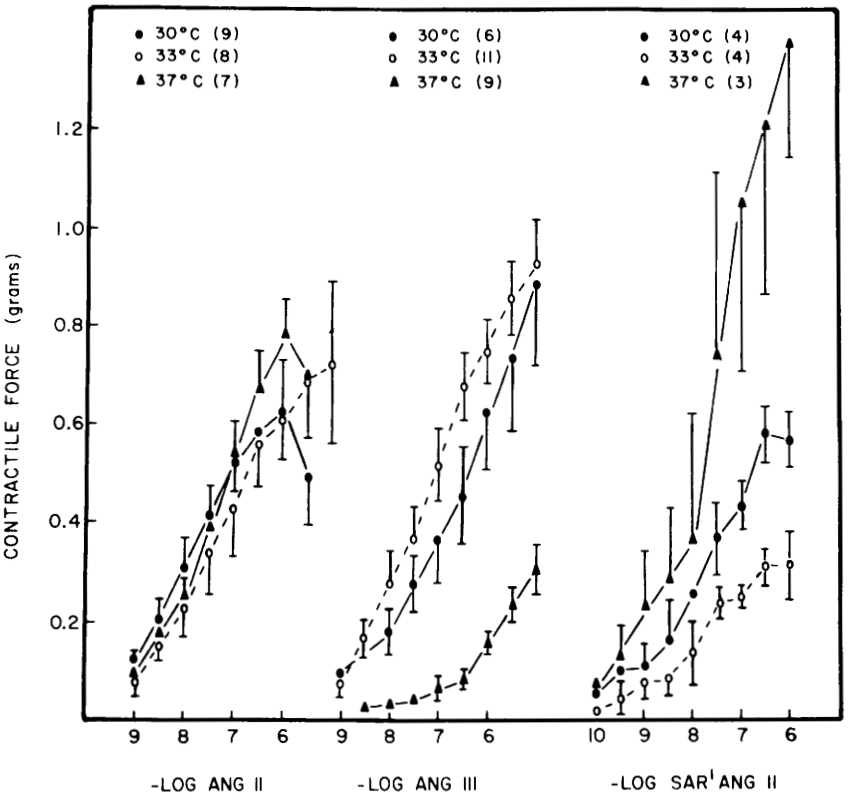

FIG. 2. Concentration–response curves for angiotensin II, angiotensin III, and sarcosyl[1]-angiotensin II at different temperatures in transmurally-stimulated atria. Points are mean values and bars represent the SEM. Numbers in parentheses indicate the number of donor animals per group. Values on the abscissa represent molar concentrations. Responses to angiotensin III at 37°C and sarcosyl[1]-angiotensin II at 33°C were significantly depressed relative to the other peptides at the same temperature ($p < 0.025$).

TABLE I. MAXIMAL RESPONSES[a] TO ANGIOTENSINS II AND III AND SARCOSYL[1]-ANGIOTENSIN II AT VARYING TEMPERATURES

Temperature (°C)	Angiotensin II	Angiotensin III		Sarcosyl[1]-angiotensin II
		Transmurally stimulated atria		
30	0.64 ± 0.10 (9)	0.91 ± 0.14 (6)		0.59 ± 0.06 (4)
33	0.72 ± 0.10 (8)	0.95 ± 0.09 (11) $P < 0.001$	$P < 0.005$	0.35 ± 0.06* (4) $P < 0.01$
37	0.81 ± 0.09 (7)	0.31 ± 0.05 (9)	$P < 0.001$	1.40 ± 0.25* (3)
		Punctate-stimulated atria		
30	0.94 ± 0.13 (7)	0.72 ± 0.13 (7)		
33	1.02 ± 0.17 (7)	0.88 ± 0.20 (6)		
37	0.81 ± 0.11 (8)	1.03 ± 0.27 (4)		
		Aortic rings		
30	1.68 ± 0.25 (4)	1.68 ± 0.16 (5)		1.03 ± 0.05 (3) $P < 0.05$
33	2.05 ± 0.17 (8)	1.85 ± 0.18 (9)		1.84 ± 0.17 (3)
37	2.09 ± 0.21 (7)	1.93 ± 0.19 (9)		2.29 ± 0.08 (3)

[a] Values are maximal responses expressed in grams of force generated ±SEM. If a clear maximum was not apparent on the concentration-response curve the response at 10^{-5} M was included as the maximum response. Numbers in parentheses are the number of preparations per group. The presence of a P value indicates a significant difference between the groups the P separates.

* Indicates a significant difference ($P < 0.05$) between responses to sarcosyl[1]-angiotensin II and angiotensin II at a given temperature.

30°C, but angiotensin II was significantly more potent at 30°C as shown in Table I. Maximal effects of angiotensin III at 30 and 33°C were similar. The order of potency at 33°C was sarcosyl[1]-angiotensin II > angiotensin II > angiotensin III with the potency ratios 3.7:1.7:1. The EC_{50} for angiotensin II was not significantly different than that for angiotensin III, indicating that these peptides are nearly equipotent at 33°C. Maximal inotropic effects of angiotensins II and III were not significantly different either (Table I). Therefore, angiotensins II and III have similar activities at this temperature.

Inotropic responses to sarcosyl[1]-angiotensin II were enhanced at 37°C. Alternatively, both the potency and the maximal response of angiotensin III were dramatically reduced at 37°C. Maximal inotropic responses to sarcosyl[1]-antiotensin II produced greater effects than angiotensin II ($P < 0.025$) or angiotensin III ($P < 0.001$) as shown in Table II. Maximal responses to sarcosyl[1]-angiotensin II were also significantly larger at 37°C than at 33 ($P < 0.01$) or 30°C ($P < 0.025$). In contrast, angiotensin III-induced elevations in contractile force at 37°C were significantly reduced at all concentrations in comparison

TABLE II. EC_{50} (-log(M)) VALUES FOR ANGIOTENSINS II AND III AND SARCOSYL[1]-ANGIOTENSIN II AT VARYING TEMPERATURES

Temperature (°C)	Angiotensin II		Angiotensin III		Sarcosyl[1]-angiotensin II
			Transmurally stimulated atria		
30	7.97 ± 0.10 (9) $P < 0.025$	$P < 0.001$	6.70 ± 0.20 (6) $P < 0.05$	$P < 0.01$	8.08 ± 0.41 (4)
33	7.55 ± 0.10 (8)		7.31 ± 0.16 (10) $P < 0.001$		7.88 ± 0.17 (4)
37	7.62 ± 0.25 (6)	$P < 0.001$	6.34 ± 0.14 (9)	$P < 0.005$	7.69 ± 0.40 (3)
			Punctate-stimulated atria		
30	7.89 ± 0.14 (7)		7.09 ± 0.38 (6)		
33	7.47 ± 0.24 (7)		6.90 ± 0.23 (6)		
37	7.61 ± 0.16 (8)	$P > 0.01$	6.59 ± 0.29 (4)		
			Aortic rings		
30	8.26 ± 0.38 (4)		7.76 ± 0.21 (5)		8.82 ± 0.60 (3)
33	8.47 ± 0.12 (8)	$P < 0.001$	7.58 ± 0.11 (8)	$P < 0.005$	8.42 ± 0.18 (3) $P < 0.05$
37	8.69 ± 0.13 (7)	$P < 0.001$	7.32 ± 0.12 (8)	$P < 0.001$	8.95 ± 0.19 (3)

Note. Values are the mean $-\log EC_{50}$ ± SEM for each group. Numbers in parentheses are the number of preparations in each group. A P value in the table indicates the level of significance among the groups it separates.

to those at 30 or 33°C ($P < 0.025$) and in comparison to responses of angiotensin II or sarcosyl[1]-angiotensin II at 37°C ($P < 0.025$). In addition to the suppressed efficacy, angiotensin III was also less potent at 37°C than at 33°C ($P < 0.001$) and was less potent than sarcosyl[1]-angiotensin II or angiotensin II. The order of potency was sarcosyl[1]-angiotensin II = angiotensin II > angiotensin III with a potency ratio of 22:22:1. These results indicated that transmurally-stimulated atria responded to an angiotensin heptapeptide, angiotensin III, in a temperature-dependent manner that was opposite to that of sarcosyl[1]-angiotensin II. Angiotensin II effects were relatively insensitive to temperature alterations.

Responses of punctate-stimulated atria to angiotensins II and III are shown in Fig. 3. Consistent responses to sarcosyl[1]-angiotensin II were not obtained in this preparation; therefore, those results were not included. Temperature changes had little influence on the efficacies of either angiotensins II or III. Angiotensin II was 10-fold more potent than angiotensin III at 37°C ($P < 0.01$) but the EC_{50} for angiotensin II was not significantly different than that for angiotensin III at 33 or 30°C (Table II). These data indicate that the influences of angiotensin on atrial force generation are far more sensitive to temperature with transmural than with punctate stimulation.

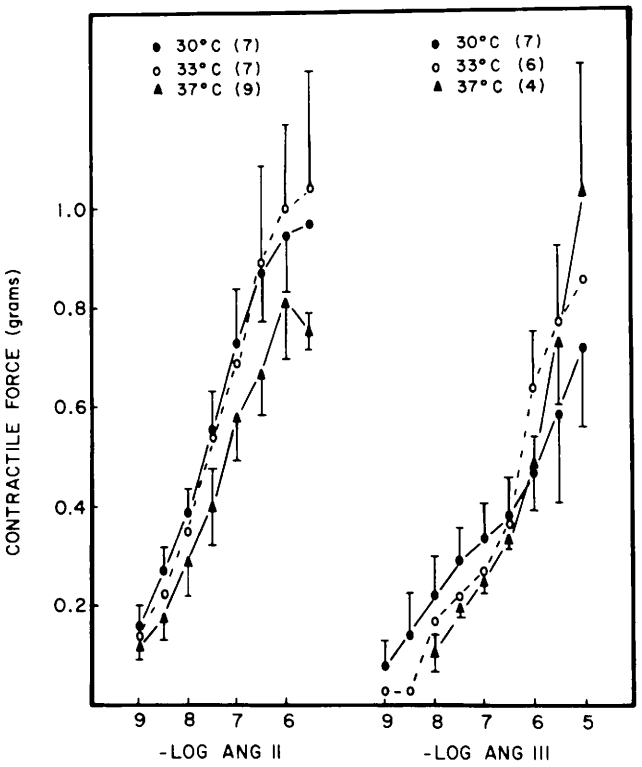

FIG. 3. Concentration–response curves for angiotensins II and III at 30, 33, and 37°C in punctate-stimulated atria. Points are mean values, bars are the SEM, and the numbers in parentheses are the number of donor rabbits per group. Values on the abscissa represent molar concentrations. No significant differences in efficacy or potency were produced by temperature changes.

Responses of aortic rings to the angiotensins are depicted in Fig. 4 and Tables I and II. Constrictor effects of angiotensin II and angiotensin III were not influenced significantly by temperature alterations. However, maximal responses to sarcosyl[1]-angiotensin II were significantly depressed at 30°C relative to 33 or 37°C ($P < 0.05$). These data support the finding that sarcosyl[1]-angiotensin II is more efficacious and potent at 37°C than at lower temperatures.

Discussion. The results of this study indicate that inotropic actions of angiotensin peptides are differentially affected by temperature changes. Responses to angiotensin II in cardiac or aortic preparations were consistent regardless of temperature except for a significant enhancement of potency at 30°C in transmurally-stimulated atria. Angiotensin III actions in transmurally-stimulated atria were sensitized and enhanced by lowering the temperature, whereas effects of sarcosyl[1]-angiotensin II in atria and aortae were reduced by lowering the temperature. For instance, angiotensin III was 58% as potent as angiotensin II in transmurally-stimulated atria at 33°C but less than 25% as potent as angiotensin II at other temperatures. Thus, small temperature changes can dramatically influence potency ratios among the angiotensin peptides.

The mechanism by which temperature changes alter potency and efficacy of the angiotensins was not investigated. Kunos and co-workers (9) have suggested that adrenergic receptors undergo a temperature-induced shift from α to β as temperature is lowered. This hypothesis has been subsequently challenged (10). A potential shift from an octapeptide- to a heptapeptide-selective receptor as temperature is lowered could explain the results of this study provided that a significant fraction of angiotensin II is converted to angio-

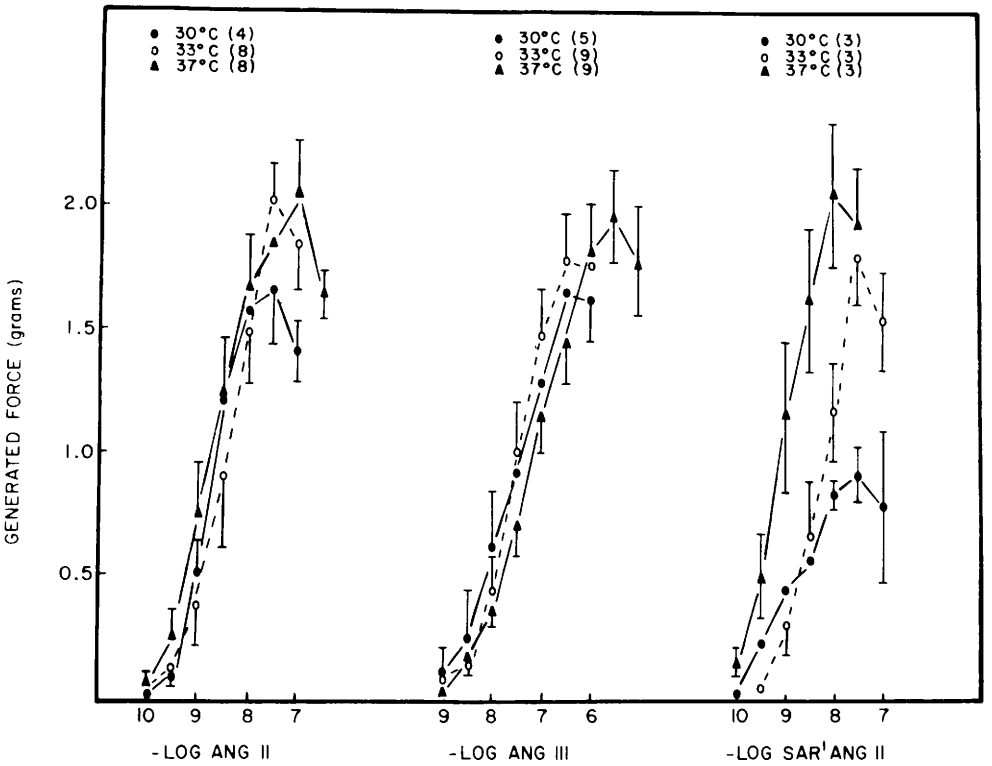

FIG. 4. Concentration–response curves for the angiotensin peptides at different temperatures in aortic rings. Points are mean values, bars are the SEM, and numbers indicate the number of donor animals per group. Values on the abscissa represent molar concentrations. The efficacy of sarcosyl¹-angiotensin II at 30°C was significantly reduced in comparison to its efficacy at 33 and 37°C ($P < 0.05$).

tensin III at the lower temperatures. A complication of this hypothesis is that separate receptors for angiotensins II and III have not been clearly identified as yet, although some antagonists exhibit some selectivity for blockade of angiotensin II or III actions (11, 12, 13).

Reduced catabolism of angiotensin III at 30 and 33°C is an unlikely explanation for its enhanced potency and efficacy in transmurally-stimulated atria. Angiotensin III actions were not affected by temperature alterations in punctate-stimulated atria but catabolizing enzymes should be affected to an equivalent degree in the two atrial preparations. Also, angiotensin II actions should have been affected by major alterations in the activity of degrading enzymes. In addition, sarcosyl¹-angiotensin II is resistant to aminopeptidases (8) and its efficacy was actually reduced at lower temperatures. Thus, temperature-induced changes in the inotropic effects of the angiotensins in heart and aortae are probably not related to changes in catabolism.

Angiotensin receptors or events following angiotensin binding to receptors could be influenced by temperature alterations. Cellular internalization of insulin (14) is lessened by a reduction in temperature. It is possible that angiotensin octapeptide agonists are more dependent on temperature-sensitive movements of angiotensin receptors for expression of their full biological activity than heptapeptide agonists. We have not observed an alteration in the binding affinity of angiotensin radioligands in cardiac membranes at temperatures as low as 12°C (Baker *et al., Circ Res* in press). Thus, alterations in binding affinities for the angiotensin peptides are unlikely explanations for the results of this study.

Regardless of the mechanism of action, the present study indicates that temperature has a major influence on both potency and efficacy of angiotensin peptides. Temperature effects also appear to be greater in preparations in which endogenous catecholamines are released (i.e., transmurally-stimulated atria) (6) than in preparations affected directly by the angiotensins (i.e., aortic rings and punctate-stimulated atria). Temperature effects on angiotensin catabolism appear to be an unlikely explanation for the mechanism by which temperature influences angiotensin actions. The heterogeneous effects of temperature on inotropic responses to angiotensin peptides are consistent with the hypotheses either that different populations of angiotensin receptors are present in the heart and aorta or that inotropic actions of the angiotensins are mediated by distinguishable mechanisms. The greater sensitivity of the transmurally-stimulated atria to alterations in temperature suggests that angiotensin receptors in sympathetic nerves may differ from those in cardiac and smooth muscle.

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