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# Angiotensin II Stimulation of 5'-Adenylic Acid Deaminase[1] (39174)

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It has been suggested that adenosine, by virtue of its vasodilating properties, is involved in the autoregulation of blood flow in striated muscle (1). In cardiac muscle, the major degradative pathway of adenosine monophosphate (AMP) involves dephosphorylation by 5'-nucleotidase to form adenosine, then formation of inosine by the action of adenosine deaminase. In skeletal muscle, deamination of AMP by adenylic acid deaminase precedes dephosphorylation and inosine formation (2). Although widely distributed in animal tissues, 5'-adenylic acid deaminase is present in highest concentrations in cardiac and skeletal muscle, where it is associated with myosin (3). The enzyme from rabbit skeletal muscle shows normal Michaelis–Menten kinetics, activation by univalent cations, stimulation by adenosine triphosphate (ATP) and by adenosine diphosphate (ADP), and inhibition by inorganic phosphate and fluoride (4, 5). Enzyme from calf brain shows activation by alkali metals and ATP, inhibition by guanosine triphosphate (other tri, di, and monophosphate nucleotides have little effect), and reversal of guanosine triphosphate inhibition by sodium. Magnesium has also been shown to increase the affinity of the ATP-stimulated enzyme for adenosine monophosphate (AMP) (6). In earlier studies, we noted that intravenous infusion of inosine monophosphate or of inosine raised blood pressure in the rat (7). This led to a study of factors regulating 5'-adenylic acid deaminase activity, including vasoactive compounds that might form part of a feedback control system of potential physiologic importance. The present report concerns stimulation of 5'-adenylic acid deaminase by angiotensin II (AII).

*Materials and Methods.* Rabbit skeletal muscle 5'-adenylic acid deaminase (55 units/mg protein), adenosine 5'-monophosphoric acid (sodium salt), and succinic acid were obtained from Sigma. The enzyme was specific for 5'-AMP and had no activity with adenosine, 2'-AMP, 3'-AMP, 3':5'-cyclic AMP, 5'-ADP, or 5'-ATP. The enzyme was prepared in 66% glycerol–0.33 *M* KCl and diluted 1:2000 for assay. 1-L-Asp-5-Val-angiotensin II was obtained from Ciba, or from the Department of Biological Standards, Mill Hill, London, diluted to $10^{-5}$ *M* with water and stored at −10°. 1-Ser-8-Ala-angiotensin II (P-113) was obtained from Norwich Pharmacal Company.

5'-adenylic acid deaminase activity was assayed spectrophotometrically by a modification of the method of Kalckar (3). The standard reaction mixture contained succinate buffer, pH 6.5, 60 m*M*; AMP, 0.15 *M*; 5'-adenylic acid deaminase, 0.0275 unit, and AII and other additions as indicated in individual experiments in a final volume of 1.0 ml, pH 6.5. The change in optical density at 265 nm was recorded on a Gilford 2400 recording spectrophotometer at 37°. Reaction velocity was determined from the initial slope and converted to micromoles of AMP deaminated per minute, dividing by 8.86, a factor incorporating the difference in molar absorption of AMP and inosine monophosphate at 265 nm.

*Results.* The dose–response curve for AII is shown in Fig. 1. Stimulation began around $10^{-9}$ *M* and peak response at $10^{-7}$ *M*. Higher concentrations resulted in less stimulation. Angiotensin I decapeptide was less potent, resulting in stimulation to lev-

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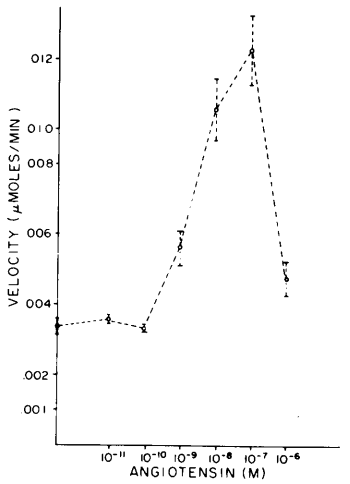


FIG. 1. Dose–response curve. The solid circle represents the mean of 56 control experiments without angiotensin II (±SE). The open circles represent the mean of six experiments containing angiotensin II. Molar concentration of angiotensin II is shown on the abscissa; velocity of 5'-adenylic acid deaminase activity is shown on the ordinate. Units of velocity are μmoles of adenosine monophosphate cleaved per minute.

els of 155% of control values at $10^{-7}$ *M*. P-113 inhibited slightly and did not block the stimulation effected by AII at equimolar concentration or when added in tenfold excess (Fig. 2).

Maximum stimulation with ATP was obtained at $10^{-5}$ *M*. The degree of stimulation imposed by ATP or by AII was approximately equal (Fig. 3). Greater stimulation could be effected by either NaCl or KCl alone. Maximum stimulation by NaCl was seen at 0.5 *M*, while maximum KCl stimulation occurred at 0.75 *M*. The addition of AII ($10^{-9}$ *M* or $10^{-7}$ *M*) resulted in additional stimulation at 0.05 and at 0.1 *M* NaCl, but not at NaCl concentration of 0.5 *M* on greater (Fig. 4), or at KCl concentration of 0.75 *M* or greater.

When dilute samples of AII were allowed to adsorb to glass by standing at room temperature overnight, the remaining solvent neither stimulated nor inhibited enzyme activity.

*Discussion.* AII is known to have a number of actions in man and in experimental animals: vasoconstriction, adrenal aldosterone secretion (8), adrenal epinephrine release (9), central nervous system stimulation (10), natriuresis, antinatriuresis (11), and direct inhibition of renin release (12). AII is formed in the pulmonary circulation and in plasma by the action of converting enzyme of angiotensin I decapeptide, extracted by a number of tissues, and de-

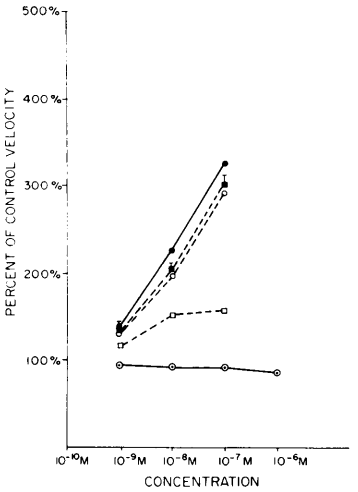


FIG. 2. A comparison of the effects of angiotensin I, angiotensin II, and 1-Ser-8-Ala-angiotensin II (P-113) on adenylic acid deaminase activity. Percent of control velocity is shown on the ordinate and molar concentration of additions on the abscissa. Control velocity was 0.0053 μmoles/min ± 0.0003 SE ($n$ = 20). ■ = angiotensin II, mean of four experiments with SE. All other points represent the results of experiments performed in duplicate, each with its own control. □ = angiotensin I; ⊙ = P-113; ● = angiotensin II plus equimolar P-113; ○ = angiotensin II plus P-113 in tenfold excess.

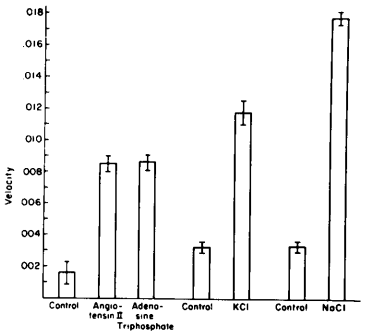


FIG. 3. Peak stimulation of 5'-adenylic acid deaminase activity by additions of angiotensin II ($10^{-7}$ *M*), adenosine triphosphate ($5 \times 10^{-5}$ *M*), KCl (0.75 *M*), or NaCl (0.5 *M*). Units of velocity are μmoles of adenosine monophosphate cleaved per minute. Each bar represents the average of six experiments (±SE).

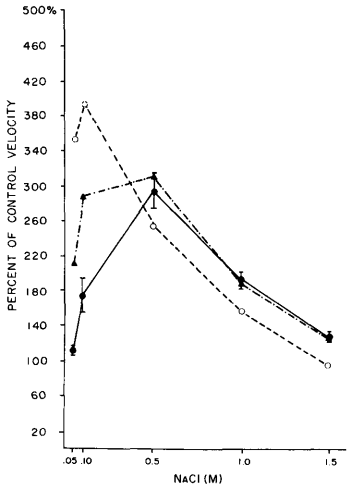


FIG. 4. Effect of sodium on stimulation of adenylic acid deaminase by angiotensin II. ● = mean of six experiments (±SE) without added angiotensin with sodium added in concentration shown on abscissa. All other points represent experiments performed in duplicate. Control velocity was .0039 μmoles/min ± 0.0002 SE ($n$ = 30). ▲ = NaCl plus angiotensin II, ($10^{-9}$ $M$); ○ = NaCl plus angiotensin II ($10^{-7}$ $M$).

stroyed by several angiotensinases. The octapeptide has been reported to be extracted during passage through the coronary circulation (13). Specific tissue binding sites for AII have been demonstrated in the adrenal gland, aorta, kidney, and uterus (14–17). The site of binding upon or within the cell remains controversial. Studies of tissue homogenates have variously placed the binding site in the nuclear (16), mitochondrial (15), and cell membrane fractions (18). Recent quantitative binding studies with radioactively labeled AII have demonstrated highly specific binding sites in the plasma membrane of vascular smooth muscle (19) and of bovine and rat adrenal cortex (20).

The present communication provides a demonstration of stimulation of an intracellular enzyme, 5′-adenylic acid deaminase, by AII. The stimulation begins at $10^{-9}$ $M$ and reaches peak effect at $10^{-7}$ $M$, concentrations approximating those required for threshold and peak response to AII for contraction of rabbit aorta and secretion of aldosterone by rat glomerulosa cells (2). In contrast, P-113, a competitive antagonist of AII, did not prevent stimulation of adenylic acid deaminase by AII, although P-113 alone was a mild inhibitor of enzyme activity in concentrations between $10^{-8}$ $M$ and $10^{-6}$ $M$. As adenylic acid deaminase is believed to be an intracellular enzyme (3), this difference may reflect differences in the structure of membrane and intracellular sites of action. Alternatively, since P-113 is known to an effective competitive inhibitor of the important physiologic effects of angiotensin II, the lack of effect in these experiments suggests that the physiologic effects of angiotensin II are not related to 5′-adenylic acid deaminase stimulation.

When adenylic acid deaminase activity was maximally stimulated by sodium or by potassium, the addition of AII did not cause further stimulation. However, maximum stimulation required 0.5 $M$ sodium or 0.75 $M$ potassium, concentrations greatly exceeding physiologic levels. At sodium or potassium concentrations approximating plasma or tissue levels, AII invariably caused additional stimulation.

Thus, angiotensin II has been reported to leave the circulation, to enter cardiac muscle cells containing relatively high 5′-adenylic acid deaminase activity, and has now been demonstrated to stimulate enzyme activity at concentrations near those required for stimulation of vascular smooth muscle or adrenal glomerulosa cells *in vitro*, although at levels higher than are ordinarily found in plasma. The physiologic significance of these observations is not clear, as the exact biologic roles of the pathways of purine salvage have not yet been established. One can postulate involvement in a chain of events linking vasuclar or cardiac muscle contraction with nucleic acid and protein synthesis necessary for muscle hypertrophy. Alternatively, elevated angiotensin II levels during hypotension might act to prevent excess production of vasodilating adenosine compounds by favoring inosine monophosphate formation.

Although the physiologic importance of these observations can only be a matter of speculation, they suggest that skeletal muscle adenylic acid deaminase might serve as a soluble indicator for study of the interactions of AII and structurally related compounds.

*Summary.* Angiotensin II has been found

to stimulate 5'-adenylic acid deaminase from rabbit skeletal muscle. Stimulation was discernible around $10^{-9}$ *M* and peak stimulation of about threefold was seen at $10^{-7}$ *M*, concentrations approximating those required for stimulation of vascular smooth muscle or adrenal glomerulosa cells. Higher concentrations produced less stimulation. Adenosine triphosphate stimulated to the same degree, but a concentration of $10^{-5}$ *M* was required for maximum stimulation, while maximum stimulation with sodium or potassium required 0.5 *M* and 0.75 *M*, respectively. Although the physiologic significance of these observations has not been established, these data suggest an intracellular role for angiotensin II.

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