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Potentiation of [^{3}H]noradrenaline accumulation in rat heart by angiotensin

There have been many reports in recent years that angiotensin interacts with the sympathetic nervous system to potentiate sympathetic activity. Conflicting reports based on experiments made on isolated tissues *in vitro* and on isolated perfused organs indicate that angiotensin inhibits (Palaič & Khairallah, 1967; Panisset & Bourdois, 1968) or has no effect upon (Thoenen, Hürlimann & Haefely, 1965; Hertting & Suko, 1966) the uptake of noradrenaline at peripheral nerve endings. Results are also conflicting for *in vivo* experiments on the effect of angiotensin on myocardial uptake of noradrenaline. Thus Buckley (1965) found no significant alteration in rat myocardial catecholamines at the end of 1 h of angiotensin infusion, but several investigators (Westfall & Peach, 1965; Peach & Ford, 1968) found an early increase in myocardial noradrenaline in the intact rabbit and cat under the influence of angiotensin, although this increase was attributed to an increase in plasma noradrenaline due to angiotensin-induced release of noradrenaline from adrenal and peripheral nerve endings (Peach & Ford, 1968).

It would appear that studies on angiotensin-noradrenaline interaction in animals with an intact nervous system may offer some advantage over *in vitro* studies, because Zimmerman (1962) has shown that vasoconstrictor response to angiotensin in the perfused hindquarters of dog is partly dependent on an intact sympathetic innervation.

Fifty experiments were made on Charles River CD male rats of approximately 200 g weight. Rats were anaesthetized with sodium pentobarbitone, which has been shown not to influence the uptake of [^{3}H]noradrenaline (^{3}H-NA) in the cat (Whitby, Axelrod & Weil-Malherbe, 1961) and has been used with the same rationale in the guinea-pig (Crout, 1964). Twenty-five control rats were injected via left lateral tail vein with 0·9% saline and 25 rats were injected with 0·1 μg angiotensin II amide (Hypertensin Ciba) in 0·9% saline. One min after the injection, 40 μCi ^{3}H-NA (6·6–8·45 Ci/mmol, New England Nuclear) was injected over a period of 10 s into the right lateral tail vein. Rats were killed by cervical dislocation exactly 1 min after the second injection and the heart was quickly dissected, killing and dissection time being standardized to 25 s. The left ventricle of each heart was trimmed and washed twice with cold saline and blotted on Whatman No. 1 filter paper. Each specimen was weighed and then ground to a fine consistency with 0·4N perchloric acid and sea sand in a mortar. Specimens were washed twice and the material centrifuged and the supernatant brought to 10 ml volume. A 1 ml aliquot was added to 14 ml of counting solution and total radioactivity was counted in a Packard

Tri-Carb liquid scintillation spectrometer. Total radioactivity was considered mainly to represent ^{3}H-NA, since it has been shown that in the guinea-pig heart the peak radioactivity at 1 min after injection of ^{3}H-NA represents 90–95% ^{3}H-NA (Crout, 1964). Control experiments and angiotensin experiments were alternated singly, and the two rats of each pair were maintained under identical conditions and were matched for equal weight. Accumulation of radioactivity in the control group of rats ($n = 25$) was $625{\cdot}6 \pm 18{\cdot}1$ nCi/g in the left ventricle, while the figure for the angiotensin-treated group ($n = 25$) was $745{\cdot}7 \pm 35{\cdot}8$ (means $\pm$ s.e.), $P < 0{\cdot}005$.

My findings are consistent with those of Westfall & Peach (1965) and Peach & Ford (1968) of increased accumulation of noradrenaline in the heart of the intact animal under the influence of angiotensin, and demonstrate further that this increase in accumulation occurs with noradrenaline from an exogenous source and that the process is not dependent upon release of the endogenous amine from adrenal and sympathetic nerve endings.

The findings in these experiments are consistent with, but not conclusive of, an increase in uptake of noradrenaline by innervated myocardium under the influence of angiotensin. One factor which must be considered but cannot be isolated in these experiments is the contribution of haemodynamic effects of angiotensin, since Hertting & Suko (1966) have demonstrated that the effect of angiotensin on contraction induced by nerve stimulation in the perfused cat spleen can be duplicated by reducing flow to the spleen. Changes in blood flow and distribution could result in altered accumulation of noradrenaline in a tissue. However, even if the increased accumulation in the myocardium were due to haemodynamic changes with no effect on uptake mechanism, an increased accumulation implies that more noradrenaline will be taken up by the nerve endings since it has been shown (Dengler, Spiegel & Titus, 1961, Iversen, 1963) that uptake of noradrenaline obeys Michaelis-Menten kinetics. Thus below saturation the rate of uptake would depend upon concentration of noradrenaline in the tissue surrounding the site of uptake.

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