References

Davies, B. N. (1966). *Nature, Lond.*, **210**, 957–958.
Day, M. D. & Rand, M. J. (1963). *J. Pharm. Pharmac.*, **15**, 221–224.
Day, M. D. & Rand, M. J. (1964). *Br. J. Pharmac. Chemother.*, **22**, 72–86.
Farmer, J. B. (1965). *J. Pharm. Pharmac.*, **17**, 640–646.
Iversen, L. L., Glowinski, J. & Axelrod, J. (1966). *J. Pharmac. exp. Ther.*, **151**, 273–284.
Levi-Montalcini, R. & Angeletti, P. U. (1962). *Int. J. Neuropharmac.*, **1**, 161–164.
Muscholl, E. & Maitre, L. (1963). *Experientia*, **19**, 658–659.
Stone, C. A., Ross, C. A., Wenger, H. C., Ludden, C. T., Blessing, J. A., Totaro, J. A. & Porter, C. C. (1962). *J. Pharmac. exp. Ther.*, **136**, 80–88.
Varma, D. R. & Benfey, B. G. (1963). *Ibid.*, **141**, 310–313.
Varma, D. R. (1967). *J. Pharm. Pharmac.*, **19**, 61–62.

Inhibition of noradrenaline uptake by angiotensin

SIR,—It has been postulated that angiotensin contracts vascular smooth muscle, in part, by releasing noradrenaline from sympathetic nerve endings (Distler, Liebau & Wolff, 1965), and Feldberg & Lewis (1964) demonstrated that angiotensin liberated noradrenaline from adrenal medulla. This effect has been used to explain the potentiation of response to sympathetic nerve stimulation after the administration of this peptide (Benelli, Della Bella & Gandini, 1964), although Hertting & Suko (1966) and Thoenen, Hürlimann & Haefely (1965) could not measure increased release of noradrenaline after angiotensin administration.

Recently we demonstrated that angiotensin prevents the uptake of noradrenaline in rat brain (Palaic & Khairallah, 1967) by acting at the level of the "membrane pump" defined by Carlsson (1966). By acting in a similar manner to cocaine, angiotensin was also postulated to block re-uptake, causing supersensitivity to noradrenaline. We have now made experiments with spleen slices and rat aortae, and compared the results with those on brain stem slices.

Female Sprague-Dawley rats (ca 200 g) were decapitated. Spleen, thoracic aorta and brain stem were rapidly removed, chilled, and 0·4 mm thick slices were prepared from spleen and brain stem. Blood vessels were carefully cleaned of extraneous fat tissue and cut spirally. Sections were incubated at 37° in 5 ml Krebs solution (6·9 g NaCl, 2·1 g $NaHCO_3$, 0·35 g KCl, 0·28 g $CaCl_2$, 0·11 g $MgCl_2$, 0·14 g Na_2HPO_4, and 2·0 glucose per litre) and aerated with oxygen 95%, carbon dioxide 5%. A duplicate section was used as control.

Tissues were first equilibrated for 10 min followed by another 30 min incubation in the presence of 0·5 μg [^{14}C]noradrenaline (specific activity 254 μc/mg). Angiotensin was added to the incubation medium at the beginning, 10 min before noradrenaline. The final concentration of angiotensin was deliberately high (100 μg in 5 ml), since spleen and brain contained high levels of angiotensin destroying enzymes. At the end of incubation, the tissue was rapidly washed twice with 0·9% saline, blotted dry and weighed. After drying overnight in an oven, the tissue was burned (Kalberer & Rutschmann, 1961) and the [$^{14}CO_2$] trapped and counted by liquid scintillation.

The amount of radioactivity taken up by the three different tissues varied, being lowest in blood vessels and highest in brain (Table 1). Since nerve endings are the usual storage sites for noradrenaline, we would like to ascribe the different levels of radioactivity to different amounts of sympathetic nerve endings in these tissues. Pease (1962) reported that aorta is relatively poor in sympathetic innervation. Angiotensin inhibited uptake of noradrenaline in the

TABLE 1. EFFECT OF ANGIOTENSIN ON NORADRENALINE UPTAKE. Tissue slices and blood vessel strips were incubated with [^{14}C]noradrenaline (0·1 μg/ml) and angiotensin (20 μg/ml). Results are expressed in counts/mg tissue; averages ± standard error of mean, and P values express degrees of significance between control and angiotensin treated tissues.

Aorta		Spleen		Brain	
Control	Angiotensin	Control	Angiotensin	Control	Angiotensin
173	113	256	83	661	257
171	121	256	114	641	273
125	86	376	86	540	502
123	53	310	129	1120	480
124	105	244	222	846	450
126	106	329	192	540	430
141	116	281	198	551	178
141		314			327
141 ± 7	100 ± 8	296 ± 8	146 ± 21	700 ± 81	362 ± 42
$P < 0.0025$		$P < 0.0005$		$P < 0.0025$	

three tissues, with a 30% inhibition in aorta and a 50% inhibition in spleen and brain slices.

Thus this inhibition of uptake of noradrenaline by angiotensin seems to be a generalized phenomenon, also occurring in isolated blood vessels. This can explain the findings of Benelli & others (1964), and also results reported by McCubbin, deMoura & others (1965). In the former case nerve stimulation releases noradrenaline. Under normal circumstances, cessation of the sympathetic effect is mainly due to re-uptake of the catecholamines. Angiotensin prevents this, thus potentiating the effect of stimulation. In the latter case, tyramine releases stored noradrenaline, which normally would be taken up again. Angiotensin again prevents this, thus potentiating the tyramine effect on blood pressure.

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References

Benelli, G., Della Bella, D. & Gandini, A. (1964). *Br. J. Pharmac. Chemother.*, **22**, 211–219.
Carlsson, A. (1966). *Pharmac. Rev.*, **18**, 541–549.
Distler, A., Liebau, H., & Wolff, H. P. (1965). *Nature, Lond.*, **207**, 764–765.
Feldberg, W. & Lewis, G. P. (1964). *J. Physiol., Lond.*, **171**, 98–108.
Hertting, G. & Suko, J. (1966). *Br. J. Pharmac. Chemother.*, **26**, 686–696.
Kalberer, F. & Rutschmann, J. (1961). *Helv. chim. Acta*, **44**, 1956–1966.
McCubbin, J. W., deMoura, R. S., Page, I. H. & Olmsted, F. (1965). *Science, N.Y.*, **149**, 1395–1396.
Palaic, D. & Khairallah, P. A. (1967). *Biochem. Pharmac.* In the press.
Pease, D. C. (1962). In *Blood Vessels and Lymphatics*, editor Abramson, D. J., p. 250, New York: Academic Press.
Thoenen, H., Hürlimann, A. & Haefely, W. (1965). *Medna pharmac. exp.*, **13**, 379–387.