Mode of action of methyldopa

SIR,—Varma (1967) has recently shown that methyldopa produces its usual antihypertensive effect in immunosympathectomized rats made hypertensive by metacorticoid treatment. The author believed this observation to be inconsistent with the hypothesis of Day & Rand (1963) that methyldopa produced its antihypertensive effect by substituting a less active "false transmitter substance" for noradrenaline in the sympathetic nervous system.

We would suggest that the results of Varma (1967) could be interpreted as confirmatory rather than contradictory to our hypothesis for the following reasons. It is known that immunosympathectomy does not completely remove the sympathetic nervous system (Levi-Montalcini & Angeletti, 1962; Iversen, Glowinski & Axelrod, 1966). This explains Varma's findings of reduced (but not abolished) myocardial catecholamine content and urinary catecholamine excretion. The mean resting blood pressure in the immunosympathectomized rats was no different from that of control animals, and thus vascular tone is presumably still under sympathetic control. Immunosympathectomized hypertensive rats showed a larger mean fall in blood pressure after methyldopa (66 mm Hg) than did control hypertensive animals (40 mm Hg), although the number of observations in Varma's experiments is small. This suggests that the vascular sympathetic innervation in immunosympathectomized rats, being more sparse than in control rats, is more susceptible to the partial sympathetic nerve block produced by methyldopa than is the sympathetic innervation in control animals.

We would also like to comment on the published work of others cited by Varma (1967) as inconsistent with the false transmitter hypothesis. It has been reported that methyldopa does not reduce the effects of sympathetic stimulation (Stone, Ross & others, 1962; Varma & Benfey, 1963). However, Day & Rand (1964) showed that methyldopa did impair responses to sympathetic stimulation in their experiments but the impairment was confined to low frequencies of stimulation. This observation has since been confirmed by Farmer (1965). It is believed that physiological impulse rates in the sympathetic nervous system are low. Varma (1967) also quoted the work of Davies (1966) as being inconsistent with our hypothesis since this worker noticed no reduction in noradrenaline output on sympathetic stimulation from the cat spleen after methyldopa. Davies (1966), however, measured "noradrenaline" output by assaying his plasma samples on the pithed rat blood pressure which we find does not differentiate noradrenaline from its α-methyl analogue. Moreover, Muscholl & Maitre (1963) showed that after methyldopa treatment sympathetic nerve stimulation in rabbit isolated perfused heart preparations released a mixture of noradrenaline and α-methylnoradrenaline. These latter workers assayed the perfusate in their experiments by a specific fluorimetric method.

We would therefore suggest that the false transmitter hypothesis to explain the antihypertensive effect of methyldopa is still tenable.

Pharmacology Department,
School of Pharmacy,
University of Aston in Birmingham,
Gosta Green, Birmingham 4.

M. D. DAY
M. J. RAND

Department of Pharmacology,
University of Melbourne,
Melbourne, Victoria, Australia.

April 9, 1967

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