The effect of amantadine on the uptake of dopamine and noradrenaline by rat brain homogenates

Amantadine hydrochloride (1-adamantanamine hydrochloride), an antiviral agent (Davies, Grunert & others, 1964) has been shown to be effective against parkinsonism (Schwab, England, & others 1969, Parkes, Calver & others, 1970). The mode of action of the drug in improving the condition of the parkinsonian patient has not been elucidated. The results of Vernier, Harmon & others (1969) suggested that, in high doses, amantadine inhibits the uptake of noradrenaline into peripheral nerve endings. It seemed reasonable to suppose that a similar action on the uptake of dopamine into central neurons might, in part, explain the anti-parkinsonian activity of amantadine. To investigate this possibility, preliminary experiments have been performed using a method based on that described by Snyder & Coyle (1969).

Fresh rat brain was homogenized in 10 volumes of oxygenated (95% O_2, 5% CO_2) modified Krebs-Henseleit buffer pH 7·2, using a "Tri-R" tissue homogenizer with a teflon pestle and a clearance of 0·15–0·23 mm at 750 rev/min. After further oxygenation, 1 ml aliquots of the homogenate were placed in small lengths of $\frac{1}{4}$ inch dialysis tubing, tied at both ends to form a sac containing an air bubble to aid mixing. The sacs were placed in stoppered test-tubes containing 5·0 ml Krebs-Henseleit buffer pH 7·2, and were incubated, with constant mixing, at 37°. At the start of each experiment, either dopamine or noradrenaline was added to the external medium, in a concentration of 1·0 pg/ml. In each case, 0·05 Ci/ml of [^{14}C]labelled amine was included in the total concentration. To measure amine uptake, the contents of the dialysis bag were first centrifuged at 48 000 *g* and 0·2 ml of the supernatant fluid was added to 15 ml of scintillation fluid (1 litre toluene: 500 ml methanol: 5 g POPOP: 0·1 g PPO). The pellet was digested in 3 ml M Hyamine solution, and 0·2 ml of the digest was added to 15 ml of scintillation fluid. The number of disintegrations in 10 min at 7° were recorded using a Packard liquid scintillation counter. While the increase in radioactivity in the supernatant, caused by passive diffusion of the amine, rapidly reached a maximum, radioactivity in the pellet reached a maximum after 2 h. Uptake of amines into the pellet was shown to be sodium dependent and temperature dependent, and was inhibited by ouabain (see Fig. 1 A and B); it was therefore assumed to involve an active process. The inclusion in the homogenate and incubating medium of various concentrations of reserpine did not reduce the radioactivity in the pellet by more than 40% and it was further assumed that the uptake which was reserpine-resistant represented active uptake into intact nerve endings.

Fig. 1A shows the effect of amantadine, added to the medium and to the homogenate, on the uptake of noradrenaline into nerve endings. A concentration of 1×10^{-4}g/ml decreased the radioactivity in the pellet by 87%. Concentration between 1×10^{-6} and 5×10^{-5} g/ml produced a 37–44% decrease.

Fig. 1B shows the effect of amantadine on dopamine uptake. All concentrations between 1×10^{-6} and 5×10^{-4} g/ml produced a similar reduction in uptake of 38–52%. A concentration of 1×10^{-3} was required to produce an inhibition of 69%.

If it is assumed that the 40% of the total uptake inhibited by reserpine represents active uptake into synaptic vesicles released by homogenization, then our experiments have not excluded the possibility that amantadine will inhibit the uptake of dopamine and noradrenaline by this process. However, it seems more likely that this reduction in radioactivity represents a non-specific action, probably on passive diffusion, since it was readily produced by widely-varying concentrations of several drugs, including reserpine and amantadine.

What does emerge clearly from our results is that, under the conditions of our experiments, high concentrations of amantadine are required to produce significant

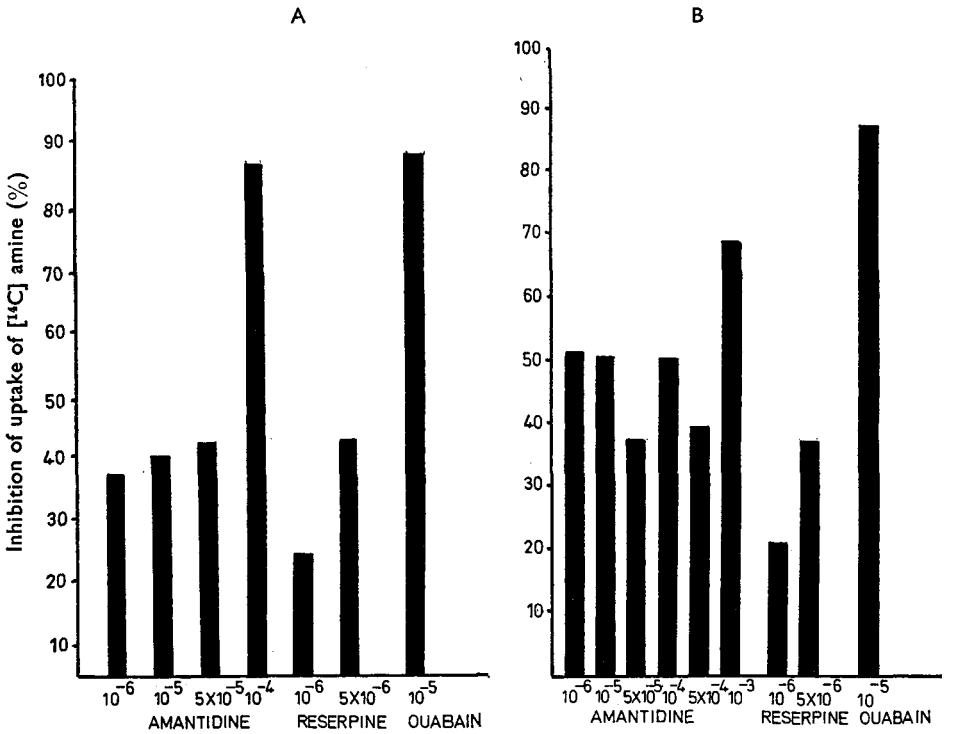

FIG. 1. The effect of amantadine, reserpine and ouabain on the uptake of: A, [^{14}C] labelled noradrenaline; B, [^{14}C] labelled dopamine by rat brain homogenates.

inhibition of noradrenaline and dopamine uptake into brain nerve-endings. In clinical use, amantadine has been found to produce side effects of central hyperactivity (e.g. insomnia, jitteriness) (Schwab & others, 1969) while Simon, Malatray & Boissier (1970) reported similar CNS stimulation in animals. Although Vernier & others (1969) found some evidence of inhibition by amantadine of noradrenaline uptake into peripheral nerve endings, the results of our experiments make it appear unlikely that the central stimulation caused by amantadine could be due to inhibition of uptake into central nerve endings. Equally, the concentration of amantadine required to produce significant inhibition of dopamine uptake suggests that this mechanism of action does not account for the clinical effectiveness of the drug against parkinsonism. This is in agreement with the report of Grelak, Clark & others (1970), who found no evidence for the inhibition by amantadine of dopamine uptake into peripheral nerve endings.

This work was supported by Geigy (U.K.) Ltd.

Pharmacology Group,
School of Pharmacy,
University of Bath,
Claverton Down,
Bath BA2 7AY
Somerset, U.K.

ELISA A. FLETCHER
P. H. REDFERN

October 8, 1970

REFERENCES

DAVIES, W. L., GRUNERT, R. R., HAFF, R. F., MCGAHEN, J. W., NEUMAYER, E. M., PAULSHOCK, M., WATTS, J. C., WOOD, T. R., HERMANN, E. C. & HOFFMAN, C. E. (1964). *Science N.Y.*, **144**, 862–863.

GRELAK, R. P., CLARK, R., STUMP, J. M. & VERNIER, V. G. (1970). *Ibid.*, **109**, 203–204.
PARKES, J. D., CALVER, D. M., ZILKHA, K. T. & KNILL, JONES, R. P. (1970). *Lancet*, **1**, 259–262.
SCHWAB, R. S., ENGLAND, A. C., POSKANZER, D. C. & YOUNG, R. R. (1969). *J. Am. med. Ass.*, **208**, 1168–1170.
SIMON, P., MALATRAY, J., & BOISSIER, J. R. (1970). *J. Pharm. Pharmac.*, **22**, 546–547.
SNYDER, S. H. & COYLE, J. T. (1969). *J. Pharmac. exp. Ther.*, **165**, 78–86.
VERNIER, V. G., HARMON, J. B., STUMP, J. M., LYNES, T. E., MARVEL, J. P., & SMITH, D. H. (1969). *Toxic. appl. Pharmac.*, **15**, 642–665.

On the mode of action of amantadine

Amantadine has been shown to have some therapeutic effect in parkinsonism, initially by Schwab, England & others (1969). In animal experiments it has been found to cause moderate central stimulation, reversal of tetrabenazine-induced sedation, a slight block of the noradrenaline uptake in the heart and to be ineffective in decreasing monoamine oxidase activity *in vitro* (Vernier, Harmon & others, 1969) An anti-cataleptic effect is reported (Simon, Malatray & Boissier, 1970; Zetler, 1970) and the drug potentiates L-dopa-induced effects in mice (Svensson & Strömberg, 1970). We have now examined some functional and biochemical aspects of the effect of amantadine on central and peripheral catecholamine neurons, and compared its mode of action with (+)-amphetamine.

We have confirmed the stimulant action of amantadine on the motor activity of mice. To investigate the effect of catecholamine depletion on this effect, amantadine HCl (150 mg/kg) was injected to female mice (strain NMRI, about 20 g) pretreated with reserpine (10 mg/kg) 5 h before. The animals were put into a test cage 90 min later, 10 min after which the motility was measured for the next 30 min by means of an Animex activity meter (Svensson & Thieme, 1969). Some mice received α-methyl-tyrosine methylester (H 44/68), an inhibitor of tyrosine hydroxylase (200 mg/kg) 15 min before amantadine, and some L-dopa (25 mg/kg) 85 min after amantadine. All injections were given intraperitoneally. Controls were given either reserpine and H 44/68 or these drugs together with L-dopa (Fig. 1). Statistical evaluation showed that amantadine caused an increased motor activity in the reserpine-pretreated mice ($P < 0.005$), which was inhibited by H44/68 ($P < 0.01$). A small dose of L-dopa, causing no motor stimulation by itself, restored the amantadine effect ($P < 0.05$). The general picture strongly resembles that of (+)-amphetamine (*c.f.* Hanson, 1966, 1967) and indicates, that amantadine requires small amounts of catecholamines for its motor stimulant effect.

For assay, amantadine HCl or (+)-amphetamine sulphate were injected in mice in various doses 105 and 45 min, respectively, before death. All mice were pretreated with reserpine (10 mg/kg) 22 h and nialamide (100 mg/kg) 4 h before death. L-Dopa (25 mg/kg) was injected subcutaneously 30 min before death. All other drugs were given intraperitoneally. Noradrenaline was determined according to Bertler, Carlsson & Rosengren (1958); dopamine according to Carlsson & Waldeck (1958, as modified by Carlsson & Lindqvist, 1962a); normetanephrine according to Carlsson & Lindqvist (1962b); methoxytyramine according to Carlsson & Waldeck (1964). Amantadine, 50 or 100 mg/kg, or (+)-amphetamine, 0·5 or 1·5 mg/kg, caused a decrease in the noradrenaline accumulation in the brain after L-dopa ($P < 0.001$). In the heart, amantadine, 50 mg/kg, caused a decrease in noradrenaline accumulation ($P < 0.025$) and so did (+)-amphetamine, 2·5 and 1·5 mg/kg, ($P < 0.001$ and < 0.01, respectively) (Table 1). No decrease of the dopamine accumulation in the brain or in the heart was found after amantadine or (+)-amphetamine but it was enhanced in the brain after 25 mg/kg amantadine ($P < 0.025$) and in the heart after 50 mg/kg ($P < 0.005$). A decrease in