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COMMUNICATIONS

Potentiation by reserpine of the inhibition by amphetamine of [^{3}H]dopamine accumulation in the rat striatum

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Amphetamine inhibits the accumulation and causes the release of dopamine in the rat striatum (Carlsson, Fuxe & others, 1966; Hamberger, 1967; Farnebo, 1971; Heikkila, Orlansky & Cohen, 1975). The question arises whether these two actions are explained by a common mechanism, i.e. if the apparent inhibition of accumulation is due to release of dopamine accumulated in the nerve terminals or if the release is due to some interaction with the membrane carrier. Pharmacological studies have indicated that amphetamine releases extravesicular dopamine, since release is not sensitive for reserpine (Carlsson & others, 1966). In fact, Farnebo (1971) observed that reserpine markedly potentiated the release of dopamine in striatal slices. It was therefore interesting to determine whether the inhibitory action of amphetamine on dopamine accumulation is also potentiated by reserpine. Cocaine was included in this study as a pure inhibitor of membrane dopamine uptake, since it is a poor releaser of dopamine (Heikkila & others, 1975).

Male Sprague-Dawley rats, 160–200 g, were used. Reserpine (5 mg kg^{-1}, i.p.) was injected one or 18 h before the experiments. The pooled striata from seven rats were homogenized in 10 volumes of ice-cold 0·24 M sucrose in a Potter-Elvehjem glass homogenizer. Cell-free homogenate was obtained by centrifugation at 800 *g* for 10 min at 0°. The accumulation of [^{3}H] dopamine-(G) (specific activity 6·2 Ci mmol^{-1}, The Radiochemical Centre, Amersham, England) was measured as described previously (Ross & Renyi, 1975). The incubation mixture in PVC centrifuge tubes contained: 100 μl of the homogenate; and 1·85 ml Krebs-Henseleit's buffer, pH 7·4; containing 0·24 mM of pargyline; 0·13mM EDTANa_2; 1·1 mM ascorbic acid; 5·8 mM D-glucose and the test compound. This mixture was incubated for 5 min at 37° or 0° before addition of 50 μl [^{3}H]dopamine (final concentration 5 × 10^{-8} M). The incubation was continued for 4 min, the tubes rapidly chilled on an ice-water bath and centrifuged at 15 000 *g* for 20 min. The washed pellets were dissolved in 1·0 ml Soluene-350 (Packard). Triplicate determinations of each concentration of the test compound were performed. The active accumulation of [^{3}H]dopamine obtained from the difference between the radioactivities in the pellet at 37° and 0° was expressed in pmol mg^{-1} protein min^{-1} incubation. Protein was determined according to Lowry, Rosebrough & others (1951) with bovine serum albumin as standard.

The accumulation of [^{3}H]dopamine was significantly ($P < 0·05$) lower in striatal homogenates from rats pretreated with reserpine 18 h before the experiments (14·5 ± 2·4 pmol mg^{-1} min^{-1}, n = 4) than in homogenates from untreated rats (23·8 ± 2·7 pmol mg^{-1} min^{-1}, n = 4). This pretreatment did not cause any significant ($P > 0·05$) change in the slope of the dose response curve of (+)-amphetamine, but shifted the curve to lower concentrations (Fig. 1). The IC50 value obtained by linear regression analysis of the log dose response curves was, in normal tissue, 4·0 × 10^{-7} M (95% confidence limits: 3·1–5·4 × 10^{-7} M) and in reserpinized tissue, 4·8 × 10^{-8} M (3·7–6·2 × 10^{-8} M), i.e. reserpine pretreatment caused an 8-fold increase in potency. To examine if the time between the injection of reserpine and the experiments influenced the result, the inhibitory potency of (+)-amphetamine was examined

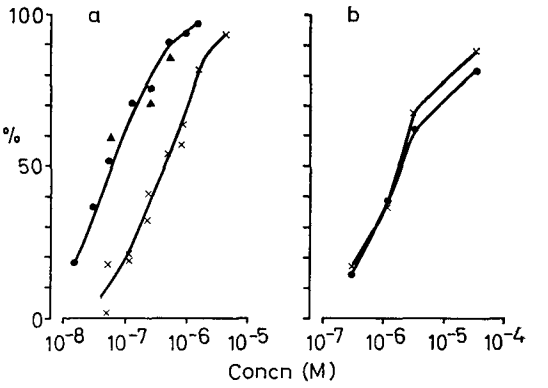

FIG. 1. Inhibition by a—(+)-amphetamine and b—cocaine of the accumulation of [^{3}H] dopamine in cell-free rat striatal homogenates. Pre-incubation for 5 min at 37° or 0° of 100 μl homogenate corresponding to 10 mg of tissue (wet weight) with the test compound in 1·85 ml Krebs-buffer, pH 7·4, containing 0·24 M pargyline, 1·1 mM ascorbic acid, 0·13 mM EDTA and 5·8 mM D-glucose. Incubation continued for 4 min with 5 × 10^{-8} M of [^{3}H] dopamine. Each point is the mean of triplicate determinations. Reserpine (5 mg kg^{-1}) was injected one or 18 h before the experiments. × Controls, ● Reserpine, 18 h. ▲ Reserpine, 1 h. Ordinate—Inhibition of accumulation (%).

1 h after reserpine. The same potentiation was obtained under this condition (Fig. 1). In contrast to amphetamine, cocaine had identical activity in normal and reserpinized preparations (IC50 = $1{\cdot}6 \times 10^{-6}$ M, 95% confidence limits: $1{\cdot}3$–$2{\cdot}0 \times 10^{-6}$ M).

The neuronal accumulation of biogenic amines is dependent on two mechanisms: membrane transport and vesicular binding (Hillarp & Malmfors, 1964). When the latter mechanism is blocked by reserpine and monoamine oxidase (MAO) is inhibited, the amine accumulated is retained in free form in the neuroplasm or bound to some extravesicular sites. If it is assumed that the membrane transport system, like several other transport systems (Schultz & Curran, 1970), is reversible, outward directed transport or efflux becomes more important for the net accumulation in the reserpinized tissues because of the elevated concentration of free amine that has accumulated in the neuroplasm (Azzaro & Smith, 1975; Ross & Kelder, 1977). This might explain the reduced dopamine accumulation observed in reserpinized striatal tissue.

Inhibition of the accumulation of biogenic amines can either be due to inhibition of the membrane carrier or to release of the amine accumulated (Ross & Renyi, 1964; Heikkila & others, 1975). Cocaine is a poor releaser of dopamine (Heikkila & others, 1975) and can therefore be presumed to inhibit membrane transport. The lack of potentiation by reserpine of the inhibitory effect of cocaine is in accordance with this view, since changes in the intraneuronal distribution of dopamine should not influence the inhibitory activity of cocaine at the membrane level.

(+)-Amphetamine appears to inhibit accumulation of dopamine by a mechanism that differs from that of cocaine, since the effect of amphetamine was markedly potentiated by reserpine. Amphetamine is a potent releaser of dopamine and this effect is also potentiated by reserpine (Farnebo, 1971). One possible explanation for the potentiation is diminished isotope dilution by endogenous dopamine, which is greatly reduced after reserpine. In the reserpinized tissue the number of [^{3}H]dopamine molecules released by amphetamine is then larger than in normal tissue, although the total number of released molecules might be the same. An alternative explanation is that in the reserpinized tissue [^{3}H]dopamine accumulated is exclusively bound to extravesicular sites to which amphetamine also has high affinity. The elevated concentration of free dopamine in the nerve terminals would then evoke an outward transport of dopamine either by passive diffusion or more likely by an active outward transport as discussed above. In the combined treatment with reserpine, an MAO inhibitor and (+)-amphetamine, this outward transport would produce an equilibrium with the uptake at a low ratio between inner and outer concentration of dopamine, which is measured as an apparent inhibition of the dopamine accumulation. If this release hypothesis is correct, the inhibitory activity of (+)-amphetamine at the dopamine membrane carrier sites is unknown and difficult to measure. Very brief incubation and addition of amphetamine together with [^{3}H] dopamine might give an estimation of this activity.

Paton (1973; 1976) proposed an alternative explanation for the effects of amphetamine with its action localized to membrane transport. According to this hypothesis, amphetamine is transported by the dopamine membrane carrier and thereby produces an accelerated exchange diffusion of dopamine, i.e. apparent dopamine release. According to this hypothesis, inhibition of dopamine accumulation is in part due to competition with dopamine for the membrane carrier and in part due to accelerated exchange diffusion. The latter is increased by reserpine because of the elevated concentration of free dopamine in the neuroplasm. An argument against this hypothesis is, however, that no clear evidence exists that amphetamine is transported by the membrane carrier (Ross, 1976).

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