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Increased responsiveness to apomorphine after REM sleep deprivation: supersensitivity of dopamine receptors or increase in dopamine turnover?*

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The behavioural effects of REM sleep deprivation in laboratory animals are characterized by increased responsiveness to external or internal stimuli (Cohen & Dement 1965; Dement 1965; Steiner & Ellman 1972; Jouvet 1977). Also, REM sleep-deprived rats show enhanced responses to apomorphine, bromocriptine and piribedil in aggressiveness, hypothermia and stereotyped behaviour (Tufik et al 1978; Tufik 1980). It has been suggested that the increased responsiveness following REM deprivation was the result of supersensitivity of post-synaptic dopaminergic receptors being brought about by the deprivation (Tufik et al 1978). Other interpretations are also possible, as, for example, an augmentation of pre-synaptic dopaminergic activity. Ghosh et al (1976) reported that striatal dopamine levels were increased by 73 and 133% after 4 and 10 days of REM sleep deprivation, respectively. Therefore, the hyperresponsiveness of deprived rats to apomorphine after deprivation could merely reflect an excess of agonistic substances at the post-synaptic receptors, that is, injected apomorphine plus the larger amount of endogenous dopamine. The present report describes experiments to clarify this.

Male albino Wistar rats, 3–4 months old, 250 to 350 g, were divided in 6 groups of 12 animals each, receiving the following treatments: groups I, II and III were normal (non-deprived) rats injected with, respectively, 50, 100 and 200 mg kg^{-1} of L-dopa; group IV had rats deprived of REM sleep for 96 h and which received 5 injections of vehicle scheduled as in group V; group V, were rats 96 h REM deprived and injected with 50 mg kg^{-1} of α-methyl-*p*-tyrosine (AMPT) daily for 5 days (the injections starting immediately before REM deprivation, the last dose being administered 6 h before the end of deprivation period); group VI, were non-deprived animals treated with AMPT as above. L-Dopa and D,L-AMPT (Sigma Lab.) were suspended in distilled water with help of Tween-80; the injections were given intraperitoneally. REM deprivation was achieved by placing the rats on 6 cm platforms surrounded by water according to Alves et al (1973).

Sixty min after injection of L-dopa and 6 h after the last dose of AMPT, which corresponded also to the end of REM deprivation period, all animals were injected i.p. with 5·0 mg kg^{-1} of freshly prepared apomorphine solution (Sigma Lab.). Pairs of identically treated animals were introduced into wire cages measuring 30 × 20 × 15 cm and the aggressive behaviour of each pair was scored in seconds for the following 30 min.

Aggressive behaviour was defined as the time animals remained upright on their hindlegs, including when one animal forced its partner to assume different patterns of submissive postures.

The results are summarized in the Fig. 1. Apomorphine failed to induce any sign of aggressive behaviour in the three groups of non-deprived rats previously treated with L-dopa; only typical stereotyped behaviour was noted in these animals. In the rats of group IV, deprived of REM sleep and injected with vehicle, apomorphine elicited 4158(701) s (mean with s.d.) of aggressive behaviour, confirming our previous finding (Tufik et al 1978). Finally, both groups pretreated with AMPT disclosed aggressive behaviour under apomorphine.

These data argue strongly against the possibility that the hyperresponsiveness to apomorphine after REM deprivation is due to an enhanced amount of dopamine which would add its effects to the injected dopaminergic agonist. If this was so, rats given L-dopa pretreatment, which enhances dopamine in brain from 40 to 130% 1 h after its injection (Masur et al 1974), should behave

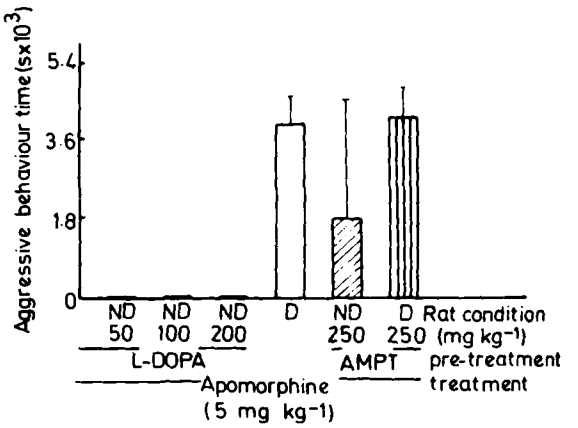

FIG. 1. Aggressive behaviour induced by 5 mg kg^{-1} of apomorphine in REM sleep deprived (D) and non-deprived (ND) after pre-treatment with L-dopa, AMPT and vehicle. The columns represent the mean of 6 pairs (with s.d.).

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aggressively after apomorphine. Furthermore, REM-deprived rats with low concentrations of brain dopamine due to blockade of its synthesis by AMPT (group V) fought intensively after receiving apomorphine. Actually, 8 h after 250 mg kg^{-1} of AMPT, brain dopamine levels were depleted respectively by 70·2 and 72·0% in controls and REM-deprived rats (Carlini et al 1977). Finally, the fact that non-deprived rats pretreated with AMPT for 5 days (group VI) displayed aggressiveness after apomorphine adds further support to the hypothesis of supersensitivity. It has been reported that in animals dopaminergic receptors develop supersensitivity as early as 24 h after treatment with AMPT (Gianutsos et al 1974), either measured through an enhanced stereotypy score elicited by apomorphine, or biochemically through dopamine and homovanilic acid levels (Costentin et al 1977). Therefore, the aggressiveness induced by apomorphine in group VI indirectly supports the hypothesis that REM deprivation induces supersensitivity of dopamine receptors, since both normal rats treated with AMPT and the REM-deprived animals presented the same aggressive response to apomorphine.

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Hypoglycaemic effect of oral insulin preparations containing Brij 35, 52, 58 or 92 and stearic acid

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Insulin administered orally with an absorption promoter, Brij-98, to 20 subjects in crossover studies by Galloway & Root (1972) gave definite responses in 10 subjects with nausea and vomiting in some cases. This has encouraged us to examine the effect of other members of the Brij series on the absorption of insulin since we have previously found Brij-58 to enhance the rectal absorption of insulin (Mesiha et al 1981). The effect of stearic acid as a lipoidal carrier for insulin in the absence and in the presence of a member of the Brij series was also studied. The blood sugar concentration response was taken as the criterion of absorption of intact physiologically active insulin.

Materials and methods

Male white rabbits of Assiut University strain (1800 ± 200 g) having normal fasting blood sugar 140–168 mg dl^{-1} were used.

Dry crystalline bovine-pork insulin (1:1) had a claimed content of 24 i.u.mg^{-1} (Minsk factory of endocrine preparations, USSR). Brij 35, 52, 58 and 92 (Atlas) were supplied by ICI United States Inc. Stearic acid powder for scientific use was from Prolabo (France).

An aqueous solution of the selected Brij (5 g/90 ml) was prepared. The accurately weighed insulin was dissolved in 1 ml of 0·01 M hydrochloric acid and mixed with 9 ml of the surfactant solution to give a final Brij concentration of 5% w/v. Control solutions were made similarly but without insulin. Insulin solution (5i.u. ml^{-1}) was prepared using no surfactant.

Turbidity occurred when insulin solution in 0·01 M hydrochloric acid was added to Brij-52 and Brij-92 solutions. The mixed solutions were used as such after thorough homogenization in a vortex mixer (Thermolyne maxi mix) for 3 min.

A melt of stearic acid (95 parts) with the appropriate Brij (5 parts) was prepared on a boiling water bath. The accurately weighed insulin crystals (5 i.u. per 100 mg base) was then added with trituration while the contents were at about 85 °C. Stirring was continued at room temperature (25 °C) until complete congealing and cooling. The granulations produced were passed through a sieve of aperture size 1·6 mm. Similar granulations were prepared with no insulin, but contain 5% surfactant and used as control. A batch was prepared containing insulin crystals dispersed in stearic acid using the same method of preparation but no surfactant was used.

Twelve rabbits were investigated in groups of four using a Latin-square design. Animals were fasted overnight before test. Solutions were given orally by syringe and stomach tube. Granulations were weighed into the dry tubes and 10 ml water injected to flush them into the stomach.

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