EFFECT OF FLUPHENAZINE ON TISSUE NORADRENALINE CONCENTRATIONS AND ITS INTERACTION WITH PARGYLINE

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Fluphenazine caused a small decrease in the noradrenaline (NA) concentrations of the rat brain, possibly by impairing granular amine storage. The drug diminished the rise in brain and heart NA concentrations induced by pargyline, suggesting that it might possess inhibitory properties on neuronal uptake mechanisms and/or NA synthesis. Fluphenazine abolished conditioned avoidance responses in rats, an effect which was maintained after the concomitant administration of pargyline, when NA concentrations remained high. This suggests that the fluphenazine-induced sedation is not mediated via its effect on brain NA content, but is possibly due to the effect of the drug on NA turnover rates in the brain.

Introduction Fluphenazine is a very potent phenothiazine antipsychotic agent (Hanlon, Michaux, Ota, Shaffer & Kurland, 1965), capable of abolishing conditioned avoidance responses (CAR) and of taming animals (High, Hassert, Rubins, Piala, Burke & Graver, 1960). Since behavioural depressions are often associated with an absolute, or relative deficiency of brain catecholamines, particularly noradrenaline (NA) (Schildkraut, 1965), it was of interest to investigate the effect of the drug on the NA concentration in the central nervous system. For the measurement of the behavioural depressions, use was made of the fact that neuroleptic drugs disrupt conditioned responses (avoidance) without necessarily blocking the unconditioned ones (escape) (Cook & Weidley, 1957). We also studied whether the behavioural depression caused by fluphenazine could, like that induced by reserpine, be effectively reversed by the monoamine oxidase (MAO) inhibitor, pargyline. The NA concentration in the hearts and brains of animals treated with a combination of both drugs was also measured.

Methods

Tissue noradrenaline concentrations Male Wistar rats (120-150 g) were divided into three groups of 24 rats each. One group received a single dose of fluphenazine dihydrochloride (Squibb), 2 mg/kg, the second group pargyline hydrochloride (Abbott) 100 mg/kg and the third group pargyline and fluphenazine simultaneously in the doses stated above. The drugs were injected subcutaneously. A further group of rats was given 0.9% w/v NaCl solution (saline) and served as controls. From each group eight rats were killed at 1 h, eight at 3 h and eight at 24 h after the treatment. The whole brain and heart were isolated. For each sample to be analysed, the hearts or brains of two rats were pooled and their total catecholamine content was estimated as 'NA' by the method of Anton & Sayre (1962).

Conditioned avoidance responses The apparatus used consisted of a four-compartment cage, with a safety area at one end and an electrifiable grid floor in the other compartments. The compartments were separated by glass panels such that the rat had to follow a zigzag path from one end of the cage (electrified) to the other (safety area). Each animal was left in the cage for 15 min to acclimatize it before conditioning. It was then placed at one end of the cage and the electric stimulator turned on. This consisted of an automatic unit, sounding a buzzer for 4 s and then delivering an electric current (60 V at a frequency of 25 Hz) for 20 seconds.

After several trials, the rat learned to respond to the sound stimulus alone, by crossing the cage to the safety area. Such animals were considered as 'conditioned', whereas those which failed to respond in this way were rejected. Ten trials were performed before drug administration allowing a 5 min rest period between the trials. Only those animals which succeeded in responding to the conditioned stimulus at least 9 times out of 10 trials were used for testing. The conditioned rats were divided into groups of 10 and their CAR was tested 1, 3 and 24 h after drug administration. The percentage of animals failing to respond to the conditioned stimulus was recorded. The drugs were injected subcutaneously in doses of 2 mg/kg for fluphenazine and 100 mg/kg for pargyline.

Results

Effect of drugs on noradrenaline concentrations As shown in Table 1, fluphenazine caused a significant reduction (44%) in brain NA concentra-

Table 1 The effect of fluphenazine (2 mg/kg, s.c.) and pargyline (100 mg/kg, s.c.) given singly or in combination, on the noradrenaline content of rat brain and heart

	Brain (μg/g wet tissue)			*Heart (μg/g wet tissue)*		
	1 h	*3 h*	*24 h*	*1 h*	*3 h*	*24 h*
Fluphenazine	0.45 ±0.03	0.234 ±0.02	0.35 ±0.033	1.39 ±0.08	1.48 ±0.18	1.13 ±0.043
Control	0.51 ±0.008	0.42 ±0.008	0.45 ±0.02	1.24 ±0.076	1.24 ±0.076	1.05 ±0.04
% change	−13.33	−44.28**	−22.20*	+12.09	+19.35	+7.62
Pargyline	0.43 ±0.045	0.89 ±0.003	1.16 ±0.024	1.75 ±0.10	2.02 ±0.05	3.70 ± 0.38
Control	0.46 ±0.03	0.41 ±0.04	0.41 ±0.041	1.59 ±0.07	1.85 ±0.15	1.75 ±0.19
% change	−6.52	+117.07**	+182.93**	+10.06	+9.19	+111.43**
Fluphenazine plus pargyline	0.59 ±0.07	0.56 ±0.006	0.84 ±0.06	1.96 ±0.06	1.92 ±0.04	2.81 ±0.26
Control	0.44 ±0.02	0.37 ±0.006	0.44 ±0.04	1.92 ±0.08	1.92 ±0.11	1.59 ±0.07
% change	+33.65	+53.15**	+90.90**	+2.08	–	+76.73**

Each value represents the mean for 8 animals ± s.e.mean.
* $P < 0.05$ compared with control; ** $P < 0.01$ compared with control.

tion, 3 h after its administration. The value tended to return to normal after 24 hours. The drug failed to show any effect on the heart NA content during the same experimental period.

Pargyline caused a progressive rise in the brain and heart NA concentration. After 24 h the values were nearly trebled or doubled respectively. The simultaneous injection of both drugs resulted in an increase in the brain NA concentrations by 50% after 2 h and 90% after 24 hours. An increase of 77% in the heart NA content was observed only after 24 hours.

Effect of drugs on conditioned avoidance responses CAR was abolished in all animals 1 h after administration of fluphenazine. This effect was maintained for 3 hours. After 24 h only 20% of the animals still showed a loss of CAR. There was no observable impairment of motor activity in the fluphenazine-treated animals. They all responded to the unconditioned stimulus. The result of the combined treatment with fluphenazine and pargyline was essentially the same as that with fluphenazine alone. All animals lost the CAR 1 h after drug administration, and the effect was still present 2 h later. After 24 h recovery occurred only in 10% of the animals which had shown a loss of the CAR.

Discussion The reduction in the brain NA concentrations caused by fluphenazine is probably due to an impairment of the storage of NA within the granules, as was previously reported for other phenothiazine derivatives (Gey & Pletscher, 1964). This may, or may not be accompanied by an inhibition of the neuronal uptake of NA (Schanberg, Schildkraut & Kopin, 1967). These two actions of phenothiazine derivatives may be mediated via their inhibitory actions on flavoenzymes (Gabay & Harris, 1966; 1967) possibly by decreasing neuronal adenosine triphosphate (ATP) content. In the heart, no change in NA content was observed, possibly as a result of a balance between the factors influencing synthesis and release. A rise in the NA content of brain and heart after pargyline has been reported by other authors (Schoepke & Wiegand, 1963; Spector, Hirsch & Brodie, 1963). This rise may be due to an increase in the endogenous NA content as a result of inhibition of intraneuronal MAO. When fluphenazine was combined with pargyline, fluphenazine diminished the rise in NA caused by pargyline alone, in both the brain and the heart. Such an interaction has been previously reported by Gey & Pletscher (1961) in the brain using chlorpromazine and other MAO inhibitors. Assuming that fluphenazine possesses an inhibitory effect on neuronal uptake of NA, as has been attributed to other phenothiazines (Schanberg *et al.*, 1967), then the increase of the endogenous NA concentration induced by pargyline could be suppressed by it.

From the behavioural experiments, it could be seen that the onset of the tranquillizing action of fluphenazine preceded the onset of the NA reduction in the brain. On combining fluphenazine with pargyline, the results show that pargyline offered no protection against the fluphenazine-induced disruption of CAR. Moore & Rech (1967) using a rotarod conditioning method have reported similar findings with fluphenazine and the MAO inhibitor, tranylcypromine. Since the net effect of such a combination was a rise in brain NA concentration, it would seem that the fluphenazine-induced sedation is not entirely dependent on the NA content of the brain. It might depend on other brain neurohumoral agents such as dopamine or 5-hydroxytryptamine. However, this seems unlikely since the breakdown of these amines is also dependent on MAO activity (Pletscher, 1966). More probably the effect is linked with changes in the turnover rates of NA (Neff & Costa, 1968). Further work is being carried out to elucidate our present findings.

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