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THE INHIBITORY EFFECT OF EIGHT NEUROLEPTIC DRUGS (BUTYROPHENONES AND PHENOTHIAZINES) ON THE RAT BRAIN NORADRENALINE INCREASE PRODUCED BY TRANYLCYPROMINE

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Abstract—The inhibitory effects of eight neuroleptics (six butyrophenones and two phenothiazines) on the penetration of noradrenaline from cytoplasm into intracellular granules in rat brain were investigated.

Four of them are potent inhibitors of this phenomenon. The results suggest that, from a physico-chemical point of view, there are two classes of neuroleptics, class I acting primarily at the intracellular granular membrane level, class II primarily at the whole cell membrane level.

IT IS generally believed that neuroleptics act by interfering with brain amines. To gather more information about the mechanism of action of these drugs, we are investigating their influence on the monoamines of rat brain under different conditions.

The particular purpose of the experiments reported in this paper was to study the action of new potent neuroleptics—the butyrophenones—on the penetration of noradrenaline (NA) from cytoplasm into intracellular granules.

The normal steady state influx-outflux can be broken in the direction of influx by artificially increasing the NA content of the cytoplasm. This was effected through the administration of tranylcypromine, a monoamine oxidase inhibitor.

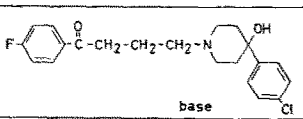
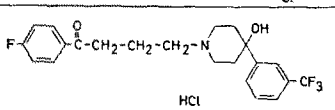
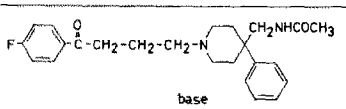
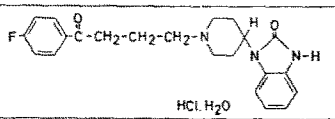
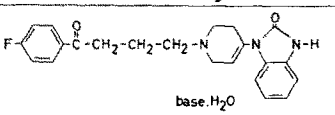
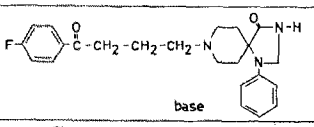
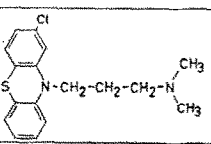
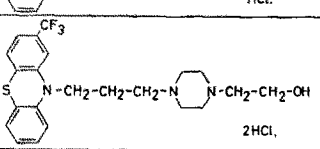
MATERIALS AND METHODS

Table 1 gives the code numbers, official names and chemical structures of the substances used. To give an idea of the relative pharmacological potency of these drugs, the activity observed in the jumping box test in rats (conditioned avoidance response) is also shown.

The figures represent the absolute and the relative values in comparison with chlorpromazine. These data were obtained by Janssen *et al.*[1–5] Clinical information about the butyrophenones can be found in the papers of Divry *et al.*[6–9] and Holderness *et al.*[10] Courvoisier *et al.*[11] and Laffan *et al.*[12] were the first to describe the pharmacological properties of chlorpromazine and fluphenazine. The method of Euler and Lishajko[13] was used for the dosage of NA. The Aluminium oxyde Woelm (neutral activity grade 1) was prepared according to De Schaepdryver.[14] The amount of fluorescent noradrenolutine was measured at 400/525 mμ respectively for activation and fluorescence in a spectrofluorimeter corrected for activation light (CGA

DC/3·000, Dr. Ciampolini, Italy). Control experiments showed, in agreement with Anton and Sayre[15] that this technique gives good recovery (95 ± 5%) and reproducible results. The values of NA are expressed in μg/g without correction. All the substances used were injected s.c. in female Wistar rats of 200 ± 10 g maintained in individual cages at a temperature of 21–23°. There was a 2-hr interval between the injection of the various neuroleptics and the injection of tranylcypromine. The rats were killed

Table 1 : Neuroleptics used

Number	Name	Chemical structure	Jumping box rats	
			ED_{50} (mg/kg)	P.R. CPZ=1
R 1625	Haloperidol	 base	0·058	15
R 2498	Triperidol	 HCl	0·025	35
R 3248	Acetabuton	 base	12·0	1/15
R4584	Benperidol	 HCl. H_2O	0·070	12
R4749	Droperidol	 base. H_2O	0·030	30
R5147	Spiroperidol	 base	0·012	75
Rhone Poulenc 4560	Chlorpromazine	 HCl.	0·92	(1)
Squibb 4918	Fluphenazine	 2HCl.	0·025	35

by decapitation exactly 1 hr after the administration of tranylcypromine. Each point on the figures represents the average of 6 dosages ± S.E. The statistical significance of differences between the rats injected with tranylcypromine only and tranylcrypomine plus neuroleptics was calculated using the non-parametric Mann–Whitney U-test.[16]

RESULTS

1. *Tranylcypromine only*

It was necessary to find out which dose of tranylcypromine produces a good increase of the brain content of NA within a short time. Figure 1 shows that 1·25 mg/kg s.c. gives a 35% increase after 1 hr ($P < 0{\cdot}001$). This dose level was chosen for the subsequent experiments.

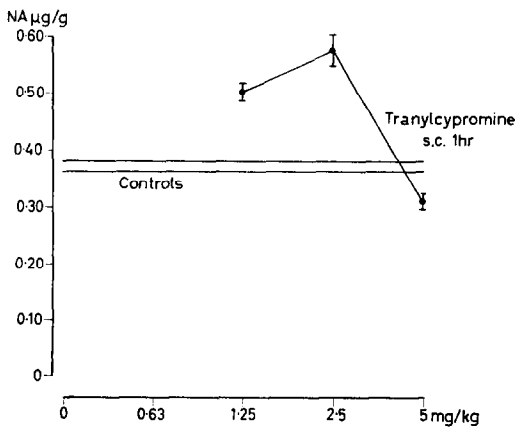


FIG. 1. Influence of various doses of tranylcypromine on the noradrenaline content of rat brain. Ordinate: noradrenaline (base) concentration in µg/g tissue. Abscissa: dose levels of tranylcypromine. The points represent the average values of 6 experiments—12 for the controls—with S.E.

2. *Tranylcypromine after neuroleptics*

Figure 2 summarizes the results obtained when tranylcypromine is injected 2 hr after the administration of different doses of neuroleptics. From this figure it appears that the activity varies greatly with the different substances. The most active—left side of the diagram—are, in order, spiroperidol (R 5147), fluphenazine, triperidol (R 2498) and haloperidol (R 1625). The right part of the diagram is occupied by much less active substances: droperidol (R 4749), benperidol (R 4584), acetabuton (R 3248) and chlorpromazine. For example, 0·31 mg/kg spiroperidol is the first dose level significantly inhibiting the NA increase normally produced by tranylcypromine. Sixteen times higher dose levels of benperidol are required to obtain the same effect.

DISCUSSION AND CONCLUSION

1. *Tranylcypromine only*

As was demonstrated by Green *et al.*[17] and many others, tranylcypromine increases the content of cerebral NA. This effect occurs with 1·25 and 2·5 mg/kg s.c. It is surprising that higher doses produce no increase. We have not studied in detail this interesting phenomenon which is also visible in the figures of Green.[17] It might be due to a releasing activity of high doses of tranylcypromine.

2. *Tranylcypromine after neuroleptics*

We shall wait until more substances have been tested before comparing our results with those of other authors. Nevertheless, some facts can already be considered. Four

of the neuroleptics chosen (class I) are potent inhibitors of the penetration of NA into intracellular granules. There is a good correlation between the pharmacological properties of these substances and the phenomena studied here. Class II is much less homogeneous and contains very weak neuroleptics such as acetabuton together with

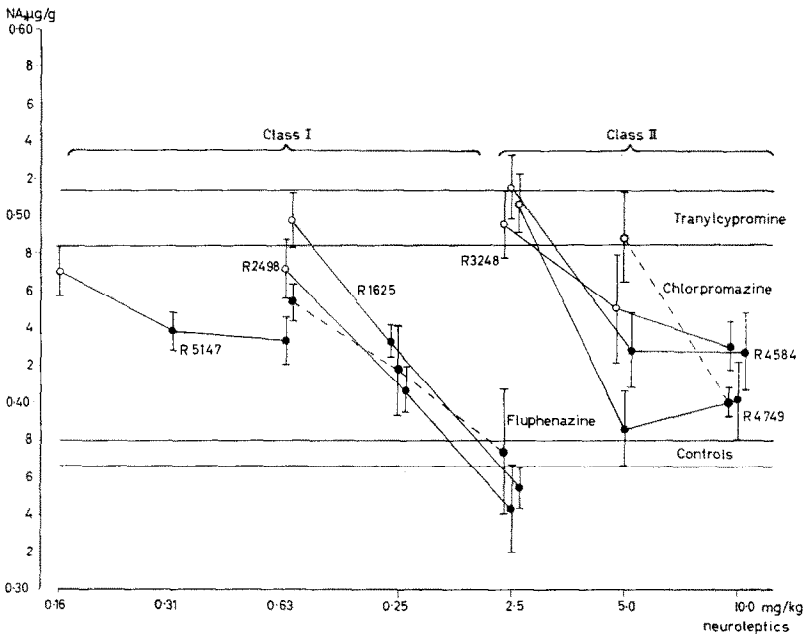


FIG. 2. Inhibitory effects of various neuroleptics on the noradrenaline increase produced in rat brain by tranylcypromine. Ordinate: noradrenaline (base) concentration in µg/g tissue. Abscissa: dose levels of neuroleptics used (salts) mg/kg s.c.; 1·25 mg/kg tranylcypromine 1·5 H_2SO_4 was administered s.c. 2 hr after the neuroleptics and 1 hr before decapitation. Each point represents an average of 6 experiments with S.E.

○ No statistical difference between neuroleptic + tranylcypromine and tranylcypromine only.
● Statistically significant difference between neuroleptic + tranylcypromine and tranylcypromine only. (P at least <0·05.)

pharmacologically potent ones such as: droperidol and benperidol. It seems therefore that a neuroleptic may also act at another level than the intracellular granular membrane, probably at the whole cell membrane level which is not investigated in our experiments. Two facts support this assumption: (1) unlike haloperidol which certainly penetrates into the cells, droperidol and benperidol are short acting; (2) unlike haloperidol both substances are potent inhibitors of the toxic effects of intravenously injected adrenaline. To summarize the first point of the discussion: we observed that potent neuroleptics such as spiroperidol, fluphenazine, triperidol and haloperidol are effective inhibitors of the penetration of NA from cytoplasm into intracellular granules. Other substances such as droperidol and benperidol may preferably act at the level of the whole cell membrane. We are now investigating this hypothesis. The second

point we want to consider in this discussion is the lack of correlation between the hypothermic effect of the neuroleptics used and their inhibitory effects on the penetration of NA into intracellular granules. In 1960, Hornykiewicz *et al.*[18] had already found evidence that chlorpromazine is able to counteract the increase of NA produced by monoamine oxidase inhibitors. This was later confirmed by Pletscher *et al.*[19] and others. More recently, Pletscher *et al.*[20] raised some doubts regarding the specificity of this phenomenon and tried to correlate the inhibition of monoamine increase in the brain with the hypothermic effect of the neuroleptics. Our results do not seem to support this hypothesis. For instance, spiroperidol fails to produce hypothermic effects at the dosages used and is thirty times more active than chlorpromazine, as an inhibitor of the tranylcypromine-induced penetration of NA into the intracellular granules. Nevertheless we must add that the experimental conditions are not the same in Pletscher's last quoted publication and in this one. The intravenously injected tryptamine must cross the cell membrane which is not examined in our experiments.

In a recent paper on haloperidol and chlorpromazine, Carlsson *et al.*[21] also demonstrate that there is no correlation between the hypothermic effects of neuroleptics and their effects on brain amines.

In conclusion, the results herein reported indicate that neuroleptics may produce their pharmacological effects by inhibiting membrane permeability at the intracellular granule or the whole cell membrane level. In our experiments, the permeability for noradrenaline was used as an 'experimental model'. It would be surprising however that noradrenaline would be the only biochemically important substance to be affected. At this stage of our knowledge and as seen by others, it is more logical to assume that the permeability to other substances, e.g. serotonine, might be similarly influenced by neuroleptic drugs.

The kind of experiment performed does not allow any conclusions as to the nature of the substance for which modification of membrane permeability is important in inducing the typical pharmacological effects obtained with neuroleptic drugs.

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