Correlation between neuroleptic-induced suppression of stereotyped behaviour and HVA concentrations in rat brain

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Among the various pharmacological effects of neuroleptics, their action as dopamine antagonists in the brain has been extensively investigated. Changes in dopamine metabolism after neuroleptics have been attributed to a blockade of dopamine receptors (Carlsson & Lindqvist, 1963, van Rossum, Boissier & others, 1970). It has been suggested that the capability of neuroleptics to enhance the dopamine turnover correlates with their antipsychotic activity (Roos, 1965; Andén, Butcher & others, 1970). Much work has been done on the neuroleptic-induced increase in the concentration of the dopamine metabolite homovanillic acid (HVA) in basal ganglia of rodents (Sharman, 1966, Andén & others, 1970; O'Keeffe, Sharman & Vogt, 1970). However, quantitative data making a comparison of HVA-increasing capacities of various neuroleptics possible are only occasionally reported (Sharman, 1966; Stille & Lauener, 1971; Wiesel & Sedvall, 1975). As we are interested in the prediction of neuroleptic activity of new compounds from animal data, it seemed worthwhile to investigate whether correlations can be found between induced changes in dopamine metabolite concentrations in rat brain and data of animal tests used for the assessment of neuroleptic potency. Determination of the HVA rise in rat corpus striatum was particularly suitable for this purpose, because a rapid and sensitive HVA assay was available (Westerink & Korf, 1975). We have measured the HVA increase after different doses of 11 phenothiazines. Dose response curves were also generated for haloperidol, pimozide and clozapine.

Solutions of phenothiazines in saline were prepared immediately before use, and ascorbic acid was added to prevent oxidation and to enhance solubility. Pimozide and clozapine were dissolved in saline with the aid of acetic acid. Male Wistar rats (180–250 g) were injected (i.p.) with the drug under investigation, while controls received a saline-ascorbic acid injection. The rats were decapitated 2 h after the injections and the corpus striatum was dissected, frozen on dry ice, weighed and homogenized in 1 ml 0·4M perchloric acid. After addition of a KOH/K-formate solution and centrifugation (15 min, 5000 *g*, 2°) the supernatant was assayed for HVA by essentially the same method as that of Westerink & Korf (1975) which is based on isolation of HVA on small Sephadex G 10 columns, followed by an automated fluorometric determination. It enabled us to measure HVA in the left and right striatal tissue independently. The recovery of the entire procedure was 81·7 ± 4·0% (n = 10). For each compound 4–6 dose levels were studied and 3–6 rats for each dose were used. Log dose response curves were constructed with measured HVA-concentration expressed as percentages of the average maximum HVA rise (9 of these are shown in Fig. 1). For virtually all compounds studied this maximum was about 5 times the control value of 0·70 ± 0·15 $\mu g\ g^{-1}$ (n = 21). From a regression line through the straight part of the curve we calculated the dose causing 50% of the maximum HVA increase (ED50% HVA) and corresponding 95% confidence limits (Table 1).

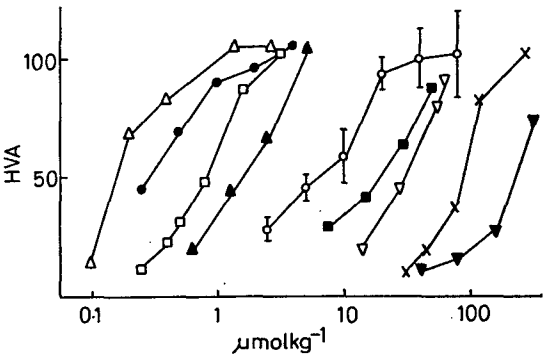

FIG. 1. Log dose-response curves of haloperidol (△–△), pimozide (●–●), thioperazine (□–□), trifluopromazine (▲–▲), chlorpromazine (○–○), perazine (■–■), thioridazine (▽–▽), clozapine (×–×) and promazine (▼–▼). HVA rise in rat striatum is expressed as percentage of the maximum HVA-increase. Each point is the mean of 3–6 determinations. Vertical bars (for clearness only shown for chlorpromazine) indicate s.e.m. of % of maximum increase.

The degree of correlation of ED50% HVA with animal data of behavioural test methods and clinical doses is shown in Table 2, the figures are Spearman rank correlation coefficients. Animal data were taken from Janssen, Niemegeers & others (1967), average clinical antipsychotic doses were obtained from Munkvad (1970), Davis (1974) and Viňar & Kršiak (1974). All doses in mg kg^{-1} were converted to μmol kg^{-1}.

The results show that all log dose-response curves have about the same slopes, thus making a comparison of relative potencies by an arbitrary measure possible. Values for the HVA increase in rat striata, measured by other authors (Bürki, Ruch & others, 1971; Stille & Lauener, 1971; Wiesel & Sedvall, 1975) indicated that we found a corresponding HVA rise at lower doses of each drug, with the exception of clozapine. This might be due, at least in some cases, to the different ways of administering the drugs.

Good correlations were found between the ED50% HVA and the lowest active doses (LAD) which antagonize apomorphine- or amphetamine-induced behavioural

Table 1. *Doses of 14 neuroleptics causing 50% of the maximum HVA increase in rat striatum (ED50% HVA, 95% confidence limits in brackets)*

Phenothiazines	μmol kg^{-1}	
1 fluphenazine	0·17	(0·12–0·24)
2 perphenazine	0·25	(0·22–0·28)
3 trifluperazine	0·34	(0·24–0·48)
4 prochlorperazine	0·48	(0·36–0·63)
5 thioperazine	0·73	(0·61–0·86)
6 triflupromazine	1·5	(1·3–1·7)
7 chlorpromazine	5·8	(4·5–7·3)
8 levomepromazine	12·4	(10·0–15·4)
9 perazine	18·1	(16·1–20·5)
10 thioridazine	28·2	(25·6–31·0)
11 promazine	200	(100–450)
Other Drugs		
12 haloperidol	0·19	(0·13–0·28)
13 pimozide	0·28	(0·27–0·30)
14 clozapine	78	(61–99)

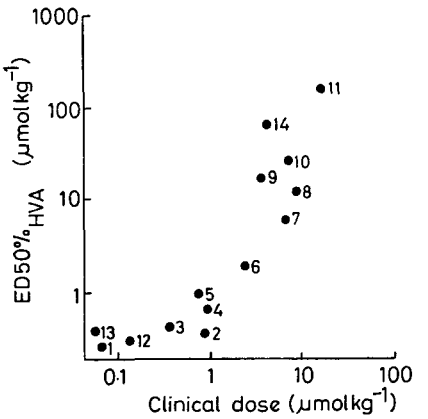

FIG. 2. Correlation between ED50% HVA and average clinical antipyschotic doses of 14 neuroleptics. Numbers refer to Table 1.

Table 2. *Correlation between animal data, clinical doses and ED50% HVA of 14 neuroleptics.* Figures are Spearman rank correlation coefficients.

LAD*–Antagonism to apomorpine stereotypy	0·94
LAD–Antagonism to apomorphine hyperactivity	0·95
LAD–Antagonism to amphetamine stereotypy†	0·96
LAD–Antagonism to amphetamine hyperactivity†	0·85
ED50–catalepsy	0·96
ED50–conditioned avoidance response	0·93
Average clinical dose	0·89

*lowest active doses.
†clozapine not included.

effects. Antagonism towards stimulation of locomotor activity correlated virtually to the same extent with ED50% HVA as did the suppression of stereotypy. If the increase in HVA concentrations reflects the dopamine receptor blocking activity of neuroleptics, then our findings are consistent with the view that amphetamine stereotypy results mainly from activation by released dopamine (Randrup & Munkvad, 1970). Hyperactivity on the other hand, is thought to be a consequence of the release of dopamine and noradrenaline (Rolinski & Scheel-Krüger, 1973). The good correlation between antagonism to hyperactivity and neuroleptic-induced HVA increase indicates that probably here too dopamine is the predominant catecholamine involved, as was recently also suggested by Khalsa & Davis (1975).

The antagonism to amphetamine- or apomorphine-induced stereotypy in rats is one of the most used tests for neuroleptic activity and is highly correlated with antipsychotic efficacy in man (van Rossum & others, 1970, Viňar & Kr˘iak, 1974). It is therefore not surprising that the ED50% also correlated rather well with average clinical antipsychotic doses (Fig. 2). Likewise it is obvious that other tests which correlate with the anti-amphetamine test (e.g. catalepsy, conditioned avoidance behaviour) also correlate with the ED50% HVA.

One of the motives for this study was the search for a simple and sensitive method for the assessment of neuroleptic activity of new phenothiazine derivatives we synthesized (Grol & Rollema, 1975). The measurement of HVA increase in rat striatum seems to be a good substitute for the determination of antagonism to stereotyped behaviour.

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A simple superfusion technique for studying release of radiolabelled 5-hydroxytryptamine from blood platelets without interference of reuptake

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Blood platelets have been suggested as an experimental model for monoamine-containing nerve endings (Sneddon, 1973). For instance, *in vitro* these organelles accumulate 5-hydroxytryptamine (5-HT) against a concentration gradient by a temperature- and sodium-dependent process, which can be described by Michaelis-Menten kinetics (Sneddon, 1969), similar to the active (re)uptake mechanisms for several neurotransmitters in nerve terminals (Iversen, 1970; Snyder, Kuhar & others, 1970). Also, with regard to the storage and release of biogenic amines, such as 5-HT, blood platelets appear to share many characteristics with nerve endings (Pletscher, Da Prada & others, 1971). Therefore many investigators have attempted to correlate changes in uptake and release processes of 5-HT in human blood platelets with disorders of the central nervous system, such as infantile autism (Bouillin, Coleman & others, 1971), depressive illness (Coppen, 1968) or migraine (Deshmukh & Harper, 1973).

Generally, studies of 5-HT release from blood platelets *in vitro* have been carried out in incubation experiments. However, the principal drawback of such methods is that both release and (re)uptake occur simultaneously. Therefore an uptake inhibition may be misinterpreted as a releasing effect and vice versa. In this communication we present a simple superfusion method for blood platelets which circumvents the problems mentioned above.

Blood (20 ml) from rats of an inbred Wistar-strain (150–200 g) was collected in centrifuge tubes, which contained 2 ml of an anticoagulant (1% EDTA in 0·9% NaCl). Erythrocytes and white cells were separated by centrifugation at 200 *g* for 15 min. The platelet-rich supernatant was removed and diluted with an equal volume of 0·9% NaCl. Then the platelets were spun down at 800 *g* for 30 min, the clear supernatant was decanted and the pellet carefully resuspended in 4 ml of Krebs-Ringer-bicarbonate (KRB) medium. The KRB-medium had the following composition (mM): NaCl (118); KCl (4·85); $CaCl_2$ (2·5); $MgSO_4$ (1·15); KH_2PO_4 (1·15); $NaHCO_3$ (25); glucose (11·1); pH 7·2–7·4. After 10 min preincubation at 37° under an atmosphere of 5% CO_2 in oxygen in a Dubnoff metabolic incubator, 5 μCi ^{3}H-5-HT was added (final concentration 1–3 $\times$ 10^{-7}M) and the incubation was continued for 15 min. Then the platelets were collected by centrifugation at 800 *g* for 30 min and the pellet was resuspended in 250 μl KRB medium. 50 μl of this suspension was applied to each of the four chambers of the superfusion system.

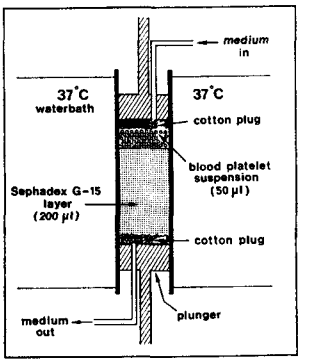

FIG. 1. Schematic drawing of one of the four superfusion chambers of the system. Basically, it consists of a syringe with two movable plungers. Well-oxygenated medium is pumped through the chambers at a rate of 0·25 ml min^{-1} and collected in 2 min fractions in vials, which are used for liquid scintillation counting of radioactivity.

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