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Comparative effects of classical and atypical antipsychotic drugs in combination with a non-amphetamine stimulant on rat brain dopamine metabolism

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Most available antipsychotic drugs produce a strong inhibition of dopamine receptors in the c.n.s. at doses comparable to clinical usage. Drugs such as haloperidol or trifluoperazine potently increase brain dopamine metabolism and turnover (Andén et al 1970; Carlsson & Lindqvist 1963), block the c.n.s. stimulation caused by amphetamine or apomorphine (Scheel-Krüger 1971) and produce both acutely and chronically extrapyramidal side effects (Parkinson-like symptoms and tardive dykinesias) in man (Baldessarini & Tarsy 1980). Clozapine, however, does not share these properties and exhibits only weak dopaminergic effects, but clozapine is an effective antipsychotic drug (Stille et al 1971; Hippius 1975). More recently, sulpiride was introduced which, also, is a weak dopamine antagonist (in striatum) and lacks extrapyramidal side effects, but is a fully effective antipsychotic drug (Justin-Besancon et al 1967; Mielke et al 1977). Not surprisingly, these two drugs have generated a great deal of research interest.

Comparisons of the effects of clinical doses of classical and atypical drugs reveal a marked difference in their ability to increase brain dopamine metabolism. The classical drugs potently increase dopamine metabolite concentrations 200-300%, at doses within the clinical range while the atypical drugs have only slight effects (Wilk et al 1975; Stawarz et al 1975; Westerink et al 1977). Some laboratories have suggested that the atypical drugs may preferentially inhibit dopamine receptors in mesolimbic and mesocortical areas compared with neostriatum. If a ratio of the percent change in these areas is determined, clozapine, sulpiride and thioridazine show a larger ratio favouring mesolimbic areas (N. accumbens or olfactory tubercle) or frontal cortex than do classical drugs (Wilk et al 1975; Westerink et al 1977). However, the absolute changes are small compared with classical neuroleptics when doses comparable with clinical drugs are used for study and the rank order of potency is the same in different brain regions (Stawarz et al 1975). Thus, the evidence for a preferential blockade of limbic and cortical dopamine receptors by clozapine or sulpiride is very weak.

The non-amphetamine c.n.s. stimulant, amfonelic acid (AFA), can be used as a sensitive tool to determine whether dopamine receptors are blocked by neuroleptic drugs. When AFA (or other non-amphetamine stimulants) is combined with a dose of haloperidol sufficient to block the hyperactivity caused by AFA (usually a minimum of 0·3 mg kg^{-1} s.c. haloperidol) a marked synergism on dopamine metabolism occurs such that dopamine metabolite concentrations increase 10 fold above control (3 fold greater than haloperidol alone; Shore 1976; McMillen 1980). Doses of haloperidol insufficient to block AFA-induced c.n.s. stimulation do not show this synergism and dopamine metabolite concentrations are less than with haloperidol alone (McMillen 1980). Apparently, a strong blockade of dopamine receptors is required to prevent the large release of dopamine by AFA from inhibiting impulse flow in order for the synergistic effect to occur (German et al 1979; Shore et al 1979). It is not known whether the same effect occurs in mesolimbic or frontal cortex areas. If AFA, in combination with classical antipsychotic drugs, does show the same interaction in frontal cortex, then combining AFA with atypical drugs may be a useful technique for comparing, in vivo, blockade of dopamine receptors in striatum versus frontal cortex.

Female Sprague-Dawley rats (Holtzman), 225–275 g, were killed in 90 min after injection with drugs, the brains rapidly removed, chilled in ice cold 0·9% NaCl (saline) and then dissected on a cold glass plate. The olfactory tubercles were removed, the brain cut and the corpus striatum removed according to Glowinski & Iversen (1966). The cortex anterior to the vertical cut through the anterior commissure was taken as the frontal cortex. The tissues were frozen over dry ice and assayed the same day for dihydroxyphenylacetic acid (DOPAC) by organic solvent extraction and fluorometric development (Murphy et al 1969).

Drugs used were: amfonelic acid (Sterling-Winthrop Research Institute, Rensselaer, N.Y.), trifluoperazine-HCl (Smith, Kline and French Laboratories, Philadelphia, PA), clozapine and thioridazine-HCl (Sandoz Pharmaceuticals, East Hanover, NJ), haloperidol (McNeil Laboratories, Ft. Washington, PA) and (+)- and (−)-sulpiride (Ravizza, Milan, Italy). All doses refer to the free acid or base.

When AFA was injected into rats, a slight increase in DOPAC concentrations occurred reaching significance in the corpus striatum. These animals exhibited intense hyperactivity and stereotypic behaviour. A previous report (McMillen 1980) demonstrated that 0·3 mg kg^{-1} s.c. haloperidol was the minimum dose which would block AFA-induced central stimulation and synergize with AFA to greatly increase DOPAC concentrations in striatum. As shown in Table 1, a similar effect occurred

Table 1. Effects of antipsychotic drugs, alone or in combination with AFA, on dopamine metabolism in rat striatum and frontal cortex. The antipsychotic drugs, with or without AFA, were administered to rats at the doses shown and the animals killed 90 min later. Numbers in parentheses after DOPAC concentrations represent the number of animals in each group.

	DOPAC $\mu g\ g^{-1} \pm$ s.e.m.			
	Striatum		Frontal cortex	
Drug (mg kg^{-1})	Drug alone	2·5 mg kg^{-1} AFA	Drug alone	2·5 mg kg^{-1} AFA
Control	1·39 ± 0·11 (12)	1·71 ± 0·23* (10)	0·19 ± 0·01 (12)	0·21 ± 0·03 (10)
Haloperidol (0·3)	5·97 ± 0·34 (8)	15·72 ± 1·38† (9)	0·57 ± 0·04 (9)	1·14 ± 0·08† (8)
Trifluoperazine (1·0)	4·49 ± 0·16 (6)	9·78 ± 0·56† (8)	0·50 ± 0·04 (6)	0·66 ± 0·04* (8)
Clozapine (20)	3·73 ± 0·23 (11)	2·23 ± 0·19† (8)	0·46 ± 0·04 (11)	0·29 ± 0·02† (8)
Clozapine + 32° (20)	4·78 ± 0·47 (8)	2·81 ± 0·21† (6)	0·56 ± 0·04 (8)	0·31 ± 0·02† (6)
Thioridazine (25)	4·04 ± 0·34 (6)	4·42 ± 0·25 (6)	0·43 ± 0·04 (6)	0·35 ± 0·03 (6)
(—)-Sulpiride (30)	2·59 ± 0·19 (9)	2·58 ± 0·18 (6)	0·40 ± 0·03 (6)	0·34 ± 0·01 (6)

* $P < 0·05$; † $P < 0·005$; different from drug alone (Student's t-test).

in the frontal cortex. Haloperidol alone produced a 3 fold increase in DOPAC concentrations in frontal cortex and a 6-fold increase when combined with 2·5 mg kg^{-1} AFA. Trifluoperazine, another potent neuroleptic drug, also synergized with AFA, although the effect was not quite so marked as with haloperidol. At this dose (1·0 mg kg^{-1} s.c.) of trifluoperazine AFA hyperactivity was completely blocked. These doses of trifluoperazine and haloperidol were approximately three fold greater than the upper range of clinical use (Usdin & Efron 1972; van Praag 1978) and experiments with the atypical antipsychotics used the same ratio for determining drug dosage. Note for comparison of drugs that the separate isomers of sulpiride were used in these studies whereas in most clinical reports the racemic mixture was used.

In Table 1 the effects of dopamine metabolism of the atypical drugs can be compared with haloperidol and trifluoperazine. Neither clozapine, thioridazine nor sulpiride synergized with AFA. AFA caused a significant reduction of the response to clozapine. Exposure of the rats to an elevated ambient temperature (20 mg kg^{-1} clozapine caused a 4–5 °C drop in body temperature) enhances the striatal DOPAC levels, but the response to AFA is the same. AFA did not have any effect on the responses to thioridazine or (—)-sulpiride. Thioridazine and clozapine partially inhibited the activity induced by AFA, but at these large doses the animals were noticeably sedated. (—)-Sulpiride, up to 100 mg kg^{-1} did not inhibit AFA-induced hyperactivity and no synergism with AFA on dopamine metabolism occurred in the striatum (Table 2). If (—)-sulpiride was injected 90 min before AFA and another 90 min allowed before killing the rats, no further change in DOPAC concentrations was observed than that seen in the 90 min experiment (Table 2). Thus, a delayed response as occurs with pimozide (McMillen et al 1980) does not occur with sulpiride. The data presented in Table 1 indicate that dopamine metabolism in both striatum and frontal cortex responded in parallel fashion to each antipsychotic drug. A preferential inhibition in frontal cortex could not be demonstrated with the AFA procedure.

Clearly, the atypical drugs are not capable of synergizing with AFA in either brain area, which suggests only a low level of dopamine receptor blockade. Smaller doses of AFA (0·5 or 1·0 mg kg^{-1}) still produce hyperactivity and do not synergize with atypical drugs (data not shown). A possible explanation of the lack of synergism with AFA is that the atypical drugs do not block presynaptic dopamine receptors and therefore cannot prevent decreased impulse flow and dopamine synthesis due to increased release of dopamine by AFA. Walters & Roth (1976) showed that clozapine has almost no blocking ability at the presynaptic autoreceptor, but that thioridazine could cause a large reversal of apomorphine inhibition in their presynaptic dopamine receptor test system. This difference between clozapine and thioridazine may explain why AFA reduces the clozapine-induced increase of DOPAC concentrations. However, a weak effect on presynaptic receptors would not explain the lack of extrapyramidal symptoms which presumably reflects postsynaptic activity.

Thus, the atypical antipsychotic drugs do not exhibit a strong in vivo blockade of dopamine receptors when administered acutely. Furthermore, there does not seem to be a very marked difference in responses of the striatal or the mesocortical dopamine neuronal systems. Whether responses to these drugs would change during chronic treatment is not clear. Although sulpiride lacks adrenergic activity, both clozapine and thioridazine are excellent α-adrenoceptor antagonists at clinical doses and these drugs may be acting by multiple receptor effects as previously suggested (McMillen & Shore 1978). Sulpiride lacks the anti-adrenergic and anti-muscarinic properties of clozapine and thioridazine which suggests that blockade of these receptors is not necessary to prevent either extrapyramidal dysfunction or the synergism with AFA. In preliminary experiments,

Table 2. Dose-response curve for the effect of sulpiride, alone or in combination with 2·5 mg kg^{-1} s.c. AFA, on rat striatal dopamine metabolism. Sulpiride was injected 90 min or 180 min (as noted) before killing the animals. AFA was always injected 90 min before killing. Numbers in parentheses represent the number of rats in each group.

	DOPAC μg g^{-1} s.e.m.	
mg kg^{-1}	alone	AFA
Saline	1·32 ± 0·08 (6)	1·71 ± 0·07* (10)
(−)-Sulpiride 10	1·77 ± 0·09 (6)	2·33 ± 0·29 (6)
30	2·44 ± 0·23 (12)	2·58 ± 0·18 (6)
100	3·08 ± 0·19 (9)	2·57 ± 0·10 (6)
30 (180 min)	2·04 ± 0·16 (6)	2·05 ± 0·09 (7)
(+)-Sulpiride 100	1·37 ± 0·13 (9)	2·12 ± 0·26* (5)

* $P < 0.05$ compared with drug alone (Student's t-test).

atropine, a very potent anti-muscarinic drug, reduced but did not prevent the increased DOPAC concentrations caused by haloperidol or haloperidol and AFA.

The introduction of clozapine and sulpiride as effective antipsychotic agents has led to the hope that effective drug therapy is possible without serious extrapyramidal side effects. Hippius (1975) noted that clozapine sat on the shelf for four years because this drug failed to elicit the 'appropriate' side effects in man and laboratory animals. This indicates how the extra-pyramidal effects have become closely tied with the desired therapeutic effect (i.e. relief of psychotic symptomatology). Thioridazine is sometimes grouped with these two drugs as constituting a class of atypical antipsychotic drugs. This may be incorrect since tardive dyskinesia has not yet been reported in patients treated with clozapine (Hippius 1975) or sulpiride (Justin-Besancon et al 1967; van Praag 1978), a claim that cannot be made for thioridazine. However, thioridazine is noted to produce less extrapyramidal side effects than other classical antipsychotic drugs (Shader & DiMascio 1970; van Praag 1978), but is not as free of these effects as are clozapine and sulpiride. Thus, its classification as either typical or atypical becomes confusing. In the present report and those of others (Wilk et al 1975; Stawarz et al 1975; Westerink et al 1977) thioridazine's effects on the dopaminergic system puts it in the atypical class of neuroleptic drugs.

That all three atypical drugs failed to synergize with AFA on dopamine metabolism suggests that this procedure may be useful for screening drugs. From Table 1, clozapine can produce a large increase in DOPAC concentration (made more marked by controlling body temperature), but does not synergize with AFA. Thus, new drugs could be tested with AFA or other dopamine uptake inhibitors in stimulant doses (nomifensine, methylphenidate, mazindole may be substituted for AFA, Shore et al 1979) to determine whether hyperactivity is blocked by the test antipsychotic drug and whether the drug combinations produce a marked increase in striatal dopamine metabolism. Failure of effective antipsychotic drugs to produce these effects would suggest a low risk of extrapyramidal side effects.

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