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Flumezapine and zotepine: 5-hydroxytryptamine antagonism not involved in the lack of synergism of these antipsychotic drugs with amfonelic acid in rats

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Two antipsychotic drugs, flumezapine and zotepine, resembled clozapine, not spiperone, in not acting synergistically with amfonelic acid to elevate striatal concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) in rats. Since flumezapine, zotepine and clozapine antagonize 5-hydroxytryptamine (5-HT) receptors with potency similar to their potency in antagonizing dopamine receptors, the possibility that 5-HT receptor blockade prevented their synergism with amfonelic acid was considered. Methiothepin, a potent 5-HT antagonist and dopamine antagonist, mimicked spiperone in causing a marked increase in striatal DOPAC in amfonelic acid-treated rats, indicating that 5-HT antagonism is not involved in the lack of synergism of flumezapine and zotepine with amfonelic acid.

McMillen (1981) suggested that antipsychotic drugs could be classified as 'classical' or 'atypical' based on their characteristics when given to rats in combination with amfonelic acid. Dopamine receptor-blocking antipsychotic drugs increase dopamine turnover in brain, causing an increase in the concentration of 3,4-dihydroxyphenylacetic acid (DOPAC), a metabolite of dopamine. Amfonelic acid, a non-amphetamine stimulant drug, has little effect on DOPAC concentration by itself but markedly potentiates the elevation of DOPAC by haloperidol, spiperone and several other antipsychotic drugs. McMillen noted that clozapine, thioridazine and sulpiride differed from haloperidol and other antipsychotic drugs in that their elevation of DOPAC was not enhanced by amfonelic acid. Since clozapine, thioridazine and sulpiride all are viewed as causing less serious extrapyramidal side effects than classical antipsychotic drugs, the failure to synergize with amfonelic acid was suggested to be a predictive test for antipsychotic drugs with low risk of extrapyramidal side effects, even though the exact mechanism of the interaction with amfonelic acid is not understood.

Waldmeier et al (1985) extended the studies of McMillen and measured striatal DOPAC elevation by additional drugs alone or in combination with amfonelic acid. These workers concluded that DOPAC elevation by drugs rated clinically as causing moderate to marked extrapyramidal side effects (benperidol, haloperidol, flupenthixol, trifluoperazine), was markedly potentiated by amfonelic acid, whereas DOPAC elevation by drugs causing weak or no extrapyramidal side effects (chlorpromazine, pimozide, pipamperone, sulpiride, clozapine, thioridazine) was weakly or not at all potentiated or even attenuated by amfonelic acid.

Here we characterize two newer antipsychotic compounds not included in the studies of McMillen (1981) or Waldmeier et al (1985), namely flumezapine and zotepine. Flumezapine (compound 9 in the paper by Chakrabarti et al 1980) was described as an antipsychotic drug candidate more potent than clozapine in blocking conditioned avoidance responding in rats. Both flumezapine and clozapine antagonized conditioned avoidance responding at doses lower than those required to produce catalepsy. These characteristics are considered predictive of clinical antipsychotic activity with low risk of extrapyramidal side effects. Zotepine is a new antipsychotic drug reported to have fewer extrapyramidal side effects in clinical studies (Satoh et al 1984).

Methods

Male Wistar rats (HSD/[WI]BR) 180–220 g (Harlan Sprague-Dawley, Inc., Cumberland, IN) were given i.p. injections of spiperone (0·5 mg kg^{-1}), clozapine (30 mg kg^{-1}), flumezapine (3 mg kg^{-1}) or zotepine (3 mg kg^{-1}) 90 min before they were killed and 5 min after amfonelic acid (2·5 mg kg^{-1} s.c.). In some rats the amfonelic acid injection was omitted, and in other rats it was given without antipsychotic drugs. Rats were decapitated, brains were quickly excised and striata were dissected and frozen on dry ice. The frozen tissue samples were stored at −15 °C before analysis of DOPAC by high performance liquid chromatography with electrochemical detection (Perry & Fuller 1979).

Results and discussion

Fig. 1 shows striatal DOPAC concentrations in rats. Spiperone, clozapine, flumezapine and zotepine all caused significant ($P < 0·05$) increases in DOPAC concentration. Amfonelic acid alone caused a slight but not statistically significant increase in DOPAC concentration. The combination of spiperone and amfonelic acid increased DOPAC concentration to more than 12 times the control level and to nearly 3 times the level in rats treated with spiperone alone. Rats treated with clozapine plus amfonelic acid had DOPAC levels lower than those treated with clozapine alone ($P < 0·05$). Our findings with spiperone and clozapine agree with those of Waldmeier et al (1985). Rats treated with flumeza-

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pine plus amfonelic acid and with zotepine plus amfonelic acid had DOPAC levels that were elevated above control values but were not significantly different from those in rats with flumezapine or zotepine, respectively, without amfonelic acid. Thus flumezapine and zotepine resemble clozapine, not spiperone, in failing to cause a synergistic rise in DOPAC when combined with amfonelic acid.

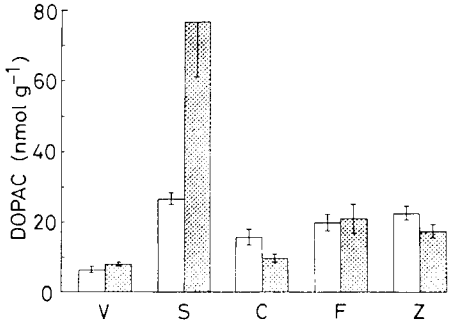

FIG. 1. Striatal DOPAC concentration in rats treated with antipsychotic drugs alone or in combination with amfonelic acid. Open bars represent rats treated i.p. with vehicle (V) (1 ml kg^{-1}), spiperone (S) (0·5 mg kg^{-1}), clozapine (C) (30 mg kg^{-1}), flumezapine (F) (3 mg kg^{-1}) or zotepine (Z) (3 mg kg^{-1}) 90 min before they were killed. Shaded bars represent rats treated in the same way 5 min after the s.c. injection of amfonelic acid (2·5 mg kg^{-1}). Mean values ± standard errors for 5 rats per group are shown. All treated groups, except that one receiving only amfonelic acid, differed significantly ($P < 0·05$) from the vehicle-treated control group.

As pointed out previously (McMillen 1981; Waldmeier et al 1985), the molecular basis for the interaction or lack of interaction between antipsychotic drugs and amfonelic acid remains unclear. The possibility was suggested (McMillen 1981) that interactions with other receptors in addition to dopamine receptors might be involved in the failure to synergize with amfonelic acid, since clozapine and thioridazine have anti-adrenergic and anti-muscarinic properties as well as being dopamine receptor antagonists. Along those lines, it is worth considering that clozapine, flumezapine and zotepine are all approximately as potent in blocking 5-hydroxytryptamine (5-HT) receptors as they are in blocking dopamine receptors, whereas spiperone is much more potent in blocking dopamine receptors than in blocking 5-HT receptors. We compared the ratio of ED50 values for these four drugs in blocking corticosterone elevation by quipazine (a 5-HT agonist) (Fuller & Snoddy 1979) or by pergolide (a dopamine agonist) (Fuller & Snoddy 1984) in rats. The ratio of ED50 for blocking quipazine divided by ED50 for blocking pergolide was 1·5 or less for flumezapine, clozapine and zotepine but was 188 for spiperone (data not shown). Blockade of 5-HT receptors has been suggested to be associated with the relative lack of extrapyramidal side effects of zotepine (Shimomura et al 1982).

Table 1. Synergistic elevation of striatal DOPAC concentration by amfonelic acid and methiothepin, a potent dopamine antagonist and 5-HT antagonist. Methiothepin (20 mg kg^{-1} i.p.) was injected 5 min after amfonelic acid (2·5 mg kg^{-1} s.c.) and 90 min before rats were killed. Mean values ± standard errors for 5 rats per group are shown.

Treatment group	Striatal DOPAC, nmol g^{-1}
Control	5·82 ± 0·33
Amfonelic acid	7·95 ± 0·65* (+36%)
Methiothepin	27·39 ± 1·96* (+370%)
Combination	95·18 ± 6·83* (+1534%)

* Significant difference from control group ($P < 0·05$).

The possibility that simultaneous block of 5-HT receptors might somehow prevent the synergistic actions between amfonelic acid and 5-HT receptor blockers is plausible in view of the findings of Waldmeier & Delini-Stula (1979) that 5-HT receptor blockers attenuate the elevation of brain DOPAC by haloperidol in rats. To examine this possibility, we studied methiothepin, a potent 5-HT antagonist and dopamine antagonist. Methiothepin antagonized quipazine-induced elevation of serum corticosterone with an ED50 of 0·21 mg kg^{-1} and antagonized pergolide-induced elevation of serum corticosterone with an ED50 of 0·22 mg kg^{-1}. Thus methiothepin is a very potent blocker both of 5-HT receptors and of dopamine receptors. Table 1 shows that methiothepin increased striatal DOPAC to more than four times the control value and that the combination of methiothepin and amfonelic acid increased striatal DOPAC to more than 16 times the control value. There was a large interaction between methiothepin and amfonelic acid, resembling the interaction that occurred with spiperone but not that with flumezapine or zotepine, indicating that 5-HT receptor blockade is unlikely to account for the failure of flumezapine and zotepine to synergize with amfonelic acid.

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