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Non-Benzodiazepine Anxiolytics: Potential Activity of Phenylpiperazines Without ^{3}H-Diazepam Displacing Action

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LLOYD, K. G., H. DEPOORTERE, B. SCATTON, H. SCHOEMAKER, B. ZIVKOVIC, PH. MANOURY, S. Z. LANGER, P. L. MORSELLI AND G. BARTHOLINI. *Non-benzodiazepine anxiolytics: Potential activity of phenylpiperazines without ^{3}H-diazepam displacing action.* PHARMACOL BIOCHEM BEHAV **23**(4) 645–652, 1985. —Four phenylpiperazine derivatives exhibited an activity similar to benzodiazepines and meprobamate in the 4-plate test. One of these (compound IV) demonstrated anxiolytic like activity in a step-down avoidance technique, in electroshock induced aggression and in the staircase test. In contrast to benzodiazepines, compound IV was not anticonvulsant, myorelaxant or sedative. Confirmation of the anxiolytic activity of compound IV in animal models was obtained in 3 separate clinical trials in anxious patients. The mechanism of action of these phenylpiperazines appears to be different from the benzodiazepines as they do not displace ^{3}H-diazepam binding nor do they interact with other elements of the GABA receptor macromolecular complex. Instead, compound IV interacts with both dopaminergic and serotoninergic neuron systems. Thus, from this data it would appear that an activity at the benzodiazepine recognition site is not obligatory for anxiolytic activity in man or in animals models.

Anxiolytic Animals models Phenylpiperazines Clinical trials

AT the present time the most effective agents for the treatment of anxiety are the benzodiazepines, and few other agents are used for this purpose [2]. These compounds have a highly specific action at a common membrane recognition site termed the benzodiazepine receptor [10, 16, 25], due to the lack of information as to the endogenous ligand for this site. In terms of physiological function, multiple lines of evidence indicate that the benzodiazepine receptor is an integral part of a large macromolecular GABA receptor complex which includes the GABA recognition site, the GABA modulated chloride ion channel and various pharmacological recognition sites, including those for benzodiazepines and barbiturates [9]. Benzodiazepines, by interacting with their specific recognition site, increase the affinity of the receptor for GABA "in vitro" and enhance the effect of GABA receptor stimulation "in vivo" [16].

Although the benzodiazepine recognition site interacts with GABA receptors, it is not clear whether this action is responsible for all of the observed effects of the benzodiazepines (e.g., anxiolytic, anticonvulsant, myorelaxant, hypnotic, etc . . .). As far as the anxiolytic effect is concerned, the evidence is inconsistent, some supporting an involvement of the benzodiazepine GABA macromolecular complex (e.g., [8, 12, 36]), other evidence suggesting that it is not involved (e.g., [18, 21, 31]). However it is likely that at least some of the undesirable effects of the benzodiazepines are related to this interaction.

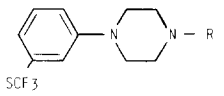
A relevant question is whether or not occupation of benzodiazepine recognition sites is obligatory for anxiolytic activity. Many non-benzodiazepine compounds interact with this site, and seem to have anxiolytic or anxiogenic potentials which parallel the qualitative changes induced in the macromolecular complex [4,9]. However GABA agonists do not exhibit anxiolytic activity except under exceptional circumstances. The anxiolytic compound meprobamate has a long history of clinical use [2] but interacts only at very high concentrations with either the benzodiazepine recognition site, or other functions of the macromolecular GABA receptor complex [24, 27, 32]. Furthermore, the new potential anxiolytic buspirone is apparently without direct activity at the benzodiazepine GABA macromolecular complex, instead having an interaction with dopamine and serotonin mediated behaviours [14, 33, 39].

We presently provide further evidence (behavioral, neurochemical, clinical) that an interaction at the benzodiazepine-GABA macromolecular complex is not obligatory for anxiolytic actions, as a new series of phenylpiperazines (Fig. 1) exerts anxiolytic effects in animals and man, yet are devoid of activity at the macromolecular complex and have a completely different neuropharmacological and neurochemical profile as compared to the benzodiazepines.

NEUROPHARMACOLOGY

The test compounds were studied in comparison with reference anxiolytics in a series of tests used to predict po-

COUPOUNDS USED



Compound #	R
I	H
II	$(CH_2)_2$ OCONH - ⬡
III	$(CH_2)_2$ OH
IV	$(CH_2)_2$ $OCOCH_3$

FIG. 1. Structures of phenylpiperazines studied.

TABLE 1

ACTIVITY OF COMPOUNDS II–IV AND REFERENCE COMPOUNDS ON ELECTROSHOCK-INDUCED AGGRESSION IN MICE

Compound	Anti-Aggression Activity ED_{50}, mg/kg PO
Compound II	20
Compound III	20
Compound IV	7
Chlordiazepoxide	15

The number of aggressive responses between two mice elicited by continuous electrical stimulation to the paws was measured as previously described [9,36]. The test compounds were administered 60 min prior to testing (3 min test period; 0.5 mA, 0.5 msec, 5 shocks/sec). n=5–10 pairs of mice per dose, 3–5 doses per dose response curve.

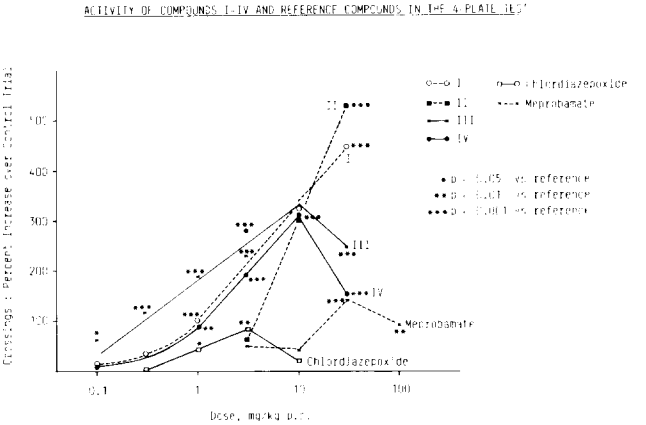


FIG. 2. The mice were trained to remain on one plate of the 4-plate apparatus as described previously [6]. On the following day the compounds were administered 60 min prior to placing the mice on the plate. The number of crossing were counted for 1 min (no electric shock) and expressed as the percent of the untreated mice; 5–15 mice per compound.

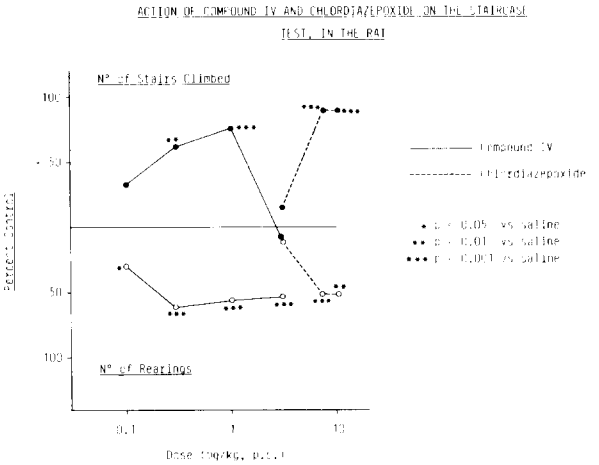


FIG. 3. The compounds were administered 60 min prior to placing the rats in the staircase apparatus. The number of rearings and stairs mounted are quantified over a period of 3 min. n=3–5 rats per dose.

tential clinical anxiolytic activity. As phenylpiperazines in general are at times associated with a decrease in food consumption (e.g., [3]), procedures associated with food or fluid intake could not be used as a reliable indicator of a potential anxiolytic activity. While this somewhat limits the spectrum of anxiolytic tests, a sufficient battery was available to compare these compounds with meprobamate and chlordiazepoxide.

All 4 piperazine derivatives (Fig. 1) plus meprobamate and chlordiazepoxide were tested in a modification of the 4-plate test [6] in which the mice (male CD-1, 18–22 g, Charles River France) were trained to remain on a 10-cm square plate, as passing from one plate to another results in an electric shock. This inhibited state in the absence of shocks is then reversed by anxiolytic compounds such as chlordiazepoxide and meprobamate (Fig. 2), for which however the maximal activity is limited by sedative/myorelaxant effects.

In the present series, compounds I–IV all exhibited an anxiolytic-like activity in the 4-plate test. The maximal effect was much greater than that of either meprobamate or chlordiazepoxide, likely due to the lack of sedative/myorelaxant effects of these compounds (see below). The minimal effective dose in this model was in the same range as the clinically active compounds: diazepam=0.1 mg/kg, PO; chlordiazepoxide=1 mg/kg, PO; meprobamate=30 mg/kg, PO; compound I=1 mg/kg, PO; compound II=10 mg/kg, PO; compound III=0.1 mg/kg, PO; compound IV=1 mg/kg, PO.

In another passive avoidance model using a step-down avoidance technique in the rat [38], at 3 mg/kg, IP, compound IV greatly diminished the latency to step-down (30% of untreated animals, $p<0.05$) whereas chlordiazepoxide did not produce a statistically significant effect at this dose (69% of untreated rats).

Compound IV was more active in decreasing electroshock induced aggression in mice [7] than was chlordiazepoxide, whereas cpds II and III were somewhat less active than this reference benzodiazepine (Table 1).

In a chamber equipped with a staircase at one end [37], benzodiazepines such as chlordiazepoxide reduce the number of rearings by rats placed in the chamber at doses which do not alter, or even increase, the number of stairs mounted on the staircase (Fig. 3). Compound IV also decreased the number of rearings at doses which did not alter (0.1 mg/kg PO), or increased (0.3 and 1 mg/kg PO) the number of stairs climbed (Fig. 3). This model was much more sensitive to compound IV (minimal effective dose=0.1

TABLE 2

EFFECT OF COMPOUNDS III AND IV AND REFERENCE COMPOUNDS IN ANTICONVULSANT TESTS IN THE MOUSE

Compound	Anti Bicuculline Convulsions ED_{50} mg/kg PO	Antipentetrazole Convulsions ED_{50} mg/kg PO	Antistrychnine Mortality ED_{50} mg/kg PO	Antielectroshock Convulsions ED_{50} mg/kg PO
III	> 30	>30	—	—
IV	>100	>30	>200	>100
Chlordiazepoxide	5	3	6	9
Diazepam	0.5	0.6	1.2	1
Meprobamate	—	30	—	—

The compounds were administered 60 min prior to the convulsant challenge, which was performed as described by Worms *et al.* [40]. The number of animals convulsing (or dead) were counted and expressed as the % of the control group. The ED_{50}'s were determined by log-probit analysis. n=10–30 mice per dose, 3–5 doses per dose response curve.

TABLE 3

COMPARISON OF COMPOUNDS I–IV AND REFERENCE COMPOUNDS IN TESTS INDICATIVE OF SECONDARY CENTRAL EFFECTS

Compound	↑ Locomotor Activity (M) ED_{50} mg/kg PO	Muscle Strength (M) ED_{50} mg/kg PO	Barbital (M) Potentiation ED_{50} mg/kg PO	Retention Deficit (R) MED mg/kg PO
I	1.0	—		—
II	10	—	—	—
III	3	>100	>100	—
IV	>100	>100	> 30	>10
Chlordiazepoxide	(↓)	7.5	5	<<10
Diazepam	(↓)	1.0	0.5	—

M=Mouse; R=Rat.

The compounds were administered 60 min prior to testing according to previously described techniques [5, 6, 11, 15]; 10–30 mice or 5–10 rats were used per dose, 3–5 doses per dose response curve.

mg/kg, PO) than to chlordiazepoxide (minimal effective dose=10 mg/kg, PO).

In a continuous-avoidance model [17], compound IV presented a profile different from that of chlordiazepoxide. Thus, the reference benzodiazepine decreased the number of avoidance responses and in parallel increased the number of shocks received (minimal effective dose=7 mg/kg, IP for both). Compound IV even at the relatively high dose of 30 mg/kg, IP, did not increase the number of shocks received.

In addition to their profiles in models predictive for a therapeutic activity, potential anxiolytics can also be compared to the benzodiazepines (and meprobamate) in tests for anticonvulsant and sedative/myorelaxant effects.

In contrast to the similarity of the phenylpiperazines to the reference anxiolytics in models for anxiolytic activity, these compounds (III,IV) did not exhibit any anticonvulsant effects in models very sensitive to the action of the benzodiazepines and meprobamate (Table 2). Furthermore, these phenylpiperazines did not show a sedative (locomotor activity, potentiation of barbital-induced sedation, loss of righting reflex) or a myorelaxant (loss of muscle strength) action and did not induce a retention deficit at doses active in the anxiolytic tests. The benzodiazepines tested (diazepam, chlordiazepoxide) were active in all of these models at doses within the "anxiolytic" dose range (Table 3).

The lack of sedative activity of compound IV is confirmed by the spectral analysis of the electrocorticogram in the curarized rat (Fig. 4), where there was an increase in the energy of the 4–6 Hz band (alerting action) whereas benzodiazepines such as flunitrazepam and nitrazepam induced generalized fast activity in the visual and sensory motor cortices (Fig. 4).

A neuropharmacological approach to the mechanism of action of anxiolytics and related compounds is via their effect on the firing rate of neurons in the dorsal Deiters nucleus. As these neurons receive a direct GABAergic input from the cerebellum [18], compounds which increase the function of the GABA-benzodiazepine macromolecular complex decrease the firing rate of the dorsal Deiters neurons [23]. Intravenous administration of diazepam and chlordiazepoxide resulted in a dose dependent decrease in the firing rate of these neurones (Fig. 5). In contrast, compound IV was inactive in this model (up to 10 mg/kg, IV) (Fig. 5), suggesting that this phenylpiperazine is devoid of activity at the GABA-benzodiazepine macromolecular complex.

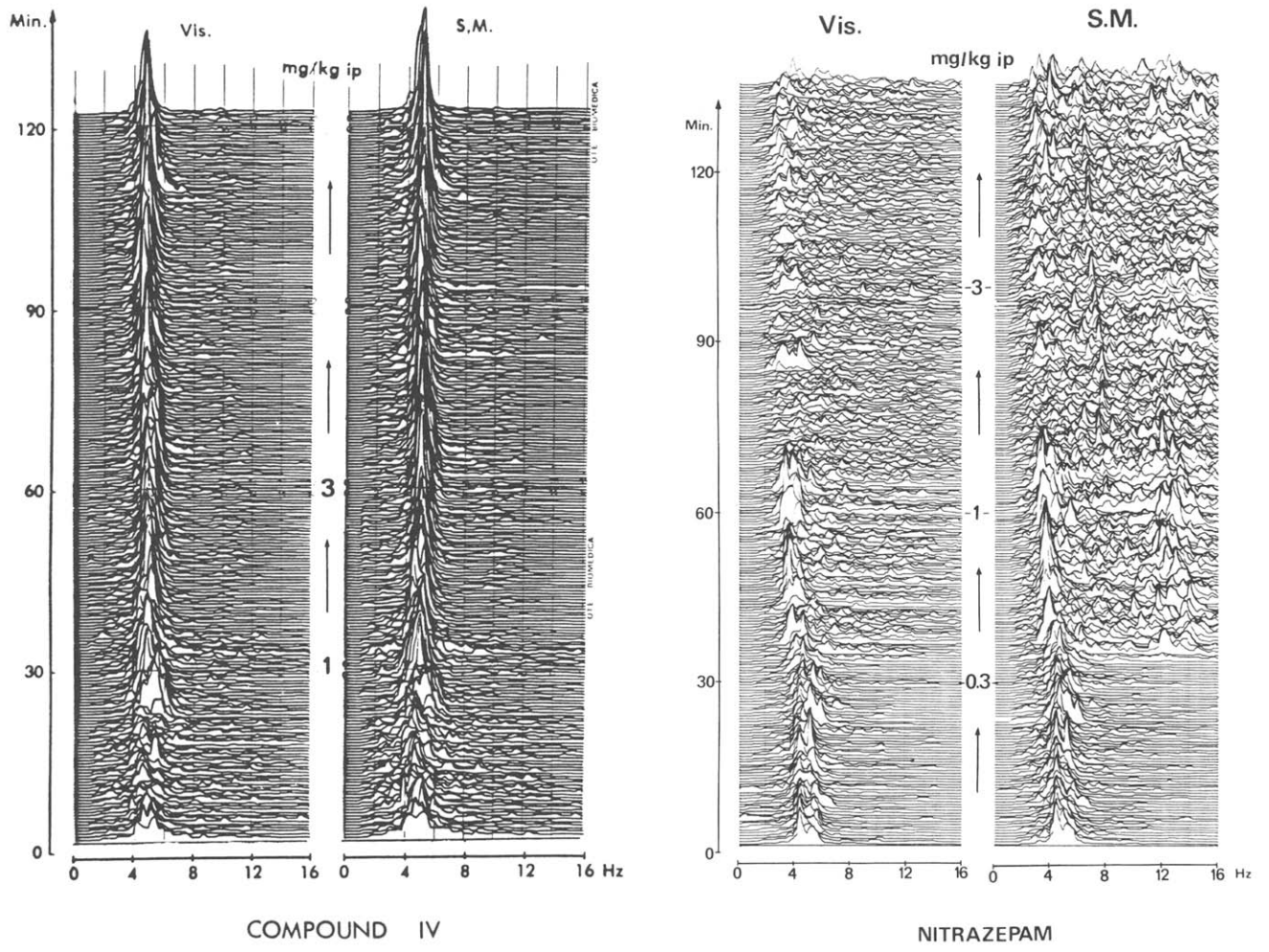


FIG. 4. Rats were implanted with electrodes in the visual (vis) and sensorimotor (S.M.) cortices, the electrocorticograms recorded and the spectral analysis performed as described previously [13]. The recordings are each from a single rat, representative of a group of 3–5 animals.

The present neuropharmacological data predict that these phenylpiperazines, especially compound IV, will have an anxiolytic effect in the clinic but that the neuropharmacological profiles will differ markedly from the benzodiazepines. These studies also suggest that the anxiolytic effect is not associated with an action at the GABA-benzodiazepine macromolecular complex. This may be a property of a certain subclass of piperazines as buspirone is also reputed to have a similar neuropharmacological profile [33].

NEUROCHEMISTRY

The binding profiles of compounds III and IV are completely different from that of the benzodiazepines (Table 4), which have a highly selective action in displacing ^{3}H-diazepam and not other radioligands [16]. The present phenylpiperazines did not displace the binding of either ^{3}H-diazepam or ^{3}H-GABA to rat cerebral membranes, thus confirming their lack of activity at the GABA-benzodiazepine macromolecular complex, as predicted from the dorsal Deiters' nucleus model (Fig. 5).

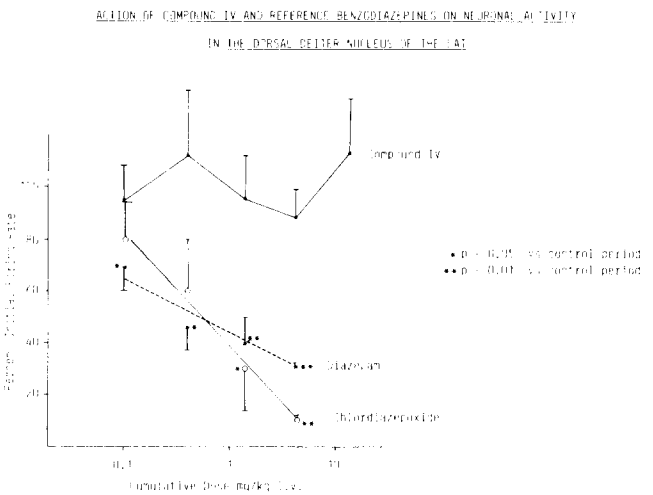


FIG. 5. Cats were implanted with multiunit electrodes in the dorsal Deiters' nucleus, and the recordings were performed as described previously [23]. n=3–5 animals per dose response curve.

TABLE 4

ACTIVITY OF COMPOUNDS III AND IV AT DIFFERENT BINDING OR RECEPTOR SITES ON RAT BRAIN MEMBRANES

Site (ligand)	Activity (IC_{50}, μM) Compound III	Compound IV	Reference Compound
Benzodiazepine (^{3}H-diazepam)	>1000	>2000	Diazepam=0.003
GABA-A (^{3}H-GABA)	>1000	>1000	GABA=0.05
Serotonin (5HT-1) (^{3}H-5HT)	—	42	Metergoline=0.008 5HT=0.006
Serotonin (5HT-2) (^{3}H-spiperone)	6.0	4.0	Methysergide=0.005 5HT=0.23
Dopamine (DA-2) (^{3}H-spiperone)	7.0	3.5	Haloperidol=0.002 Apomorphine=20
Muscarinic (^{3}H-QNB)	90	75	Atropine=0.001
β-Adrenoceptor (^{3}H-DHA)	> 500	> 500	Propranolol=0.01
α-Adrenoceptor (^{3}H-prazosin)	2.2	2.5	Prazosin=0.0028

Membranes were prepared from the rat brain and the binding assays were performed as described in Lloyd *et al.* [22]; the results are the mean of a minimum of 3 experiments.

TABLE 5

NEUROCHEMICAL PROFILE OF COMPOUND IV IN COMPARISON TO CHLORDIAZEPOXIDE

Parameter	Compound IV Dose mg/kg	Compound IV %control	Chlordiazepoxide Dose mg/kg	Chlordiazepoxide % control
Homovanillic Acid (R)				
Striatum	10, PO	132 ± 17	10, PO	81 ± 5*
Limbic System	10, PO	171 ± 20*	10, PO	76 ± 13
Dopamine Levels (R)				
Striatum	10, IP	106 ± 4	—	—
Limbic System	10, IP	113 ± 6	—	—
Dopamine Turnover (R)				
Striatum	30, IP	97 ± 3	10, PO	100 ± 2
Dopamine Release				
Striatal Slice (R)	10 μM	112 ± 19		
Striatal Push Pull Cannula (C)	1, IV	53 ± 13*	1, IV (diazepam)	135 ± 23
Norepinephrine Levels (R)				
Limbic System	10, IP	98 ± 9	—	—
Norepinephrine Turnover (R)				
Whole Brain	30, IP	129 ± 5*	30, PO	91 ± 4
Serotonin Levels (R)				
Limbic System	10, IP	95 ± 4	—	—
5-Hydroxyindoleacetic Acid Levels (R)				
Limbic System	10, IP	66 ± 3*	—	—

The levels of the monoamines, their metabolites, and their release turnover were estimated as described by Scatton *et al.* [30]. R=Rat, C=Cat.

The results are expressed as means with SEM, for 5–10 animals per group.

*$p<0.05$ vs. controls.

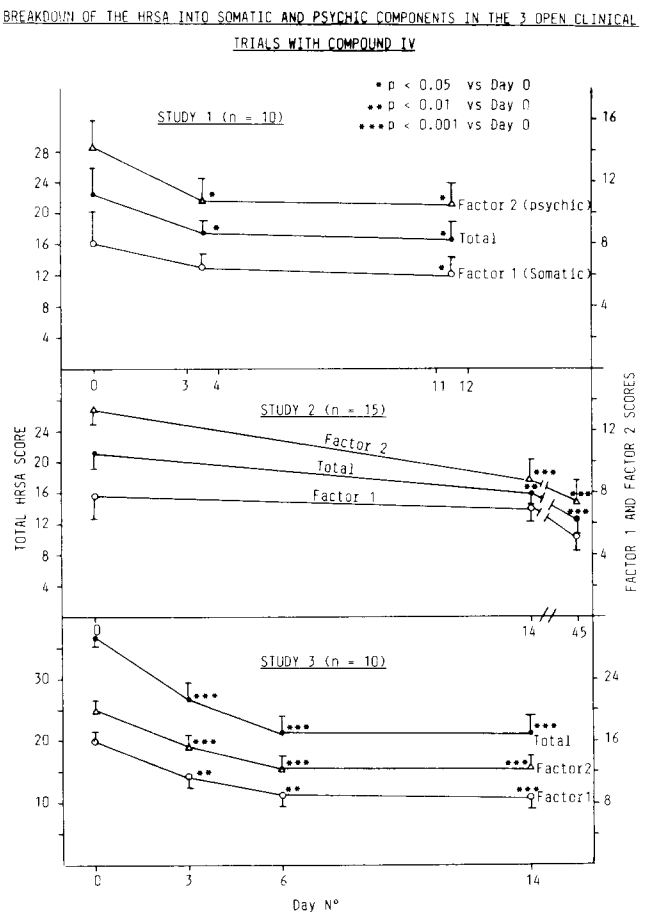


FIG. 6. See text for details of the individual clinical trials.

These compounds did however have an activity at serotonin (especially 5HT-2), dopamine and alpha adrenoceptors (Table 4). None of these activities are potent in terms of an antagonist profile, however these receptor affinities are well within the agonist range.

The weak affinity of compound IV for these different neurotransmitter receptors is reflected by the moderate alteration seen in the neurochemical indices of monoaminergic neuron function. The only modification of dopamine neuron function observed (Table 5) was an increase in limbic (but not striatal) homovanillic acid levels in the rat and a decrease in DA release into the push-pull cannula perfusate of the cat striatum. In contrast, chlordiazepoxide decreased striatal homovanillic acid levels and did not alter the release of DA into the push-pull cannula perfusate. Although this neurochemical profile of compound IV has aspects consistent with both dopamine receptor blockade (increased homovanillic acid levels) and dopamine agonist activity (decreased release of dopamine into the push-pull cannula perfusate), the overall "in vivo" activity is that of a dopamine agonist. Thus, compound IV reverses haloperidol-induced catalepsy in rats with and ED_{50} (1 mg/kg, PO) in the dose range active in anxiolytic tests. Anxiolytics of the benzodiazepine class do not reverse haloperidol catalepsy, and may even potentiate it [20].

In a manner consistent with its binding profile (Table 4), compound IV also influenced the neurochemical indices of noradrenergic and serotoninergic neuron activity (Table 5). Norepinephrine turnover was increased and 5-hydoxyindoleacetic acid levels decreased. This is consistent with a weak alpha adrenoceptor blockade and a serotonin agonist action, respectively.

This neurochemical profile of compound IV parallels that of its behaviour profile in that it is distinct from that of the benzodiazepines, with a suggestion of a dopaminergic and/or serotoninergic component. The lack of activity at the benzodiazepine recognition site is consistent with the low sedative/myorelaxant activity of compound IV and with its inactivity in different anticonvulsant tests. Furthermore, these findings are consistent with the suggestion that an activity at the GABA-benzodiazepine macromolecular complex (as identified by ^{3}H-diazepam or ^{3}H-GABA binding, or by the activity on dorsal Deiters neurons) is not obligatory for an anxiolytic activity in certain animal models.

This suggestion is reinforced by the activity reported for buspirone, another piperazine derivative with anxiolytic properties [14,33]. Buspirone does not displace ^{3}H-diazepam binding [14, 33, 39], although an interaction with the GABA-benzodiazepine macromolecular complex cannot be completely excluded as this compound has been reported to enhance ^{3}H-flunitrazepam binding "in vivo" [26,39]. In parallel with compound IV, buspirone antagonises neuroleptic induced catalepsy, increases homovanillic acid levels in the rat striatum and displaces dopamine and serotonin receptor binding [14, 33–35]. Thus, both compound IV and buspirone exert a broad spectrum modulation of central monoaminergic neurons.

CLINICAL ANXIOLYTIC ACTIVITY

Three separate open inpatient clinical trials in anxiety have been performed with compound IV administered orally (Fig. 6). In each trial, compound IV was the only anxiolytic agent provided. Only male patients entered the clinical trials.

In the first trial of 10 patients, compound IV was administered at a dose of 100–200 mg per day. At the end of 12 days, one case had an excellent response, 4 had good and 1 had moderate responses. In 2 cases the compound was inactive and 2 cases could not be evaluated. The mean onset of a clinically significant anxiolytic activity was 7 days; with a statistically significant effect occurring already at 3–4 days. As shown in Fig. 6, both the total Hamilton Rating Scale for Anxiety (HRSA) and the psychic component decreased in parallel, with the somatic compound responding slightly later.

The second trial studied 15 patients over a period of 8 weeks. This group was subdivided into 3 cases of depressive anxiety, 3 of reactional anxiety and 9 cases of neurotic anxiety. The effective dose range was from 25–75 mg/per day. In 10 of the 15 cases the treatment was successful (1 excellent, 9 moderate) 2 were weak or unresponsive and the other 3 cases dropped out of the study for diverse reasons. As can be seen from Fig. 6, a clear anxiolytic action was noted at day 14 (the first day of analysis) and persisted up to day 45. The psychic component of anxiety seemed to have responded more rapidly than the somatic component, although the total HRSA was significantly ($p<0.01$) decreased by day 14. In these patients with a combined anxiety-depression, an antidepressant action of compound IV was also noted.

The third trial consisted of 10 severe anxious patients, with analysis on days 0, 3, 6 and 14. The active dose appeared to be about 200 mg per day. At day 6, 8 cases had a good response and the other two did not respond to the treatment. The onset of action was 2–5 days, and both the

psychic and somatic components, as well as the total HRSA score, were improved. In two cases an antidepressant activity was also observed.

Thus, out of 30 patients completed in 3 open trials, there were 2 excellent responses, 22 with a moderate-good responses and 6 treatment failures. This suggests an anxiolytic activity for compound IV. In studies 1 and 2, a more rapid and marked action on the psychic than on the somatic component of the HRSA was observed. Furthermore, in none of these studies was a sedative/myorelaxant effect noted, clearly differentiating compound IV from the benzodiazepines.

GENERAL DISCUSSION

The preclinical and clinical profiles of these phenylpiperazine derivatives, especially compound IV, are those of an anxiolytic compound. However, this is the only similarity to the benzodiazepines demonstrated by compound IV. Thus, in both man and animals compound IV is devoid of sedative or myorelaxant effects. In contrast, such actions are integral components of the spectrum of action of benzodiazepines. This suggests that the mechanism of action of these phenylpiperazines is substantially different from that of the benzodiazepines. This is confirmed by the inability of these compounds to displace ^{3}H-diazepam from the highly specific benzodiazepine recognition site. Together with the divergent profile of compound IV as compared to benzodiazepines on other receptor binding sites and on the neurochemical indices of central monoamine neuron activity, it is evident that the two classes of compounds have different mechanisms of action.

These data, together with those for another piperazine derivative buspirone [14, 33–35, 39] and for meprobamate [24, 27, 32] suggest that an anxiolytic activity can be achieved without an obligatory activity at the GABA/benzodiazepine macromolecular complex. In fact, these data suggest that both a dopaminergic and serotoninergic component contribute to the anxiolytic activity of the piperazine derivatives. In this regard, a serotoninergic component in the mechanism of action of benzodiazepines has been considered possible by some authors [1, 19, 28, 29].

In conclusion, the present series of phenylpiperazines demonstrate a clear anxiolytic activity in man and in animal models. This activity is not paralleled by anticonvulsant, myorelaxant or sedative effects, nor by an action at the components of the GABA-benzodiazepine macromolecular complex. Thus, a preclinical and clinical anxiolytic effect does not seem "a priori" to be associated with an action at the benzodiazepine recognition site.

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