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Effects of gepirone, an aryl-piperazine anxiolytic drug, on aggressive behavior and brain monoaminergic neurotransmission*

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Summary. Gepirone (BMY 13805), a buspirone analog, was used to determine the antianxiety mechanism of the aryl-piperazine class of drugs. Because of the weak effects of these drugs on conflict behavior, isolation-induced aggressive mice were used as the antianxiety model. Gepirone, like buspirone, potently inhibited attacks against group housed intruder mice (ED_{50} = 4.5 mg/kg i.p.) without causing sedation or ataxia. Inhibition of aggression was potentiated by co-administration of 0.25 mg/kg methiothepin or 2.5 mg/kg methysergide. Gepirone had variable effects on dopamine metabolism and reduced 5-hydroxytryptamine (5HT) metabolism about one third after a dose of 2.5 mg/kg. In contrast to buspirone, which markedly increased dopaminergic impulse flow, gepirone inhibited the firing of most cells recorded from the substantia nigra zona compacta in doses of 2.3–10 mg/kg i.v. and the effects were reversible by administration of haloperidol. The common metabolite of buspirone and gepirone, 1-(2-pyrimidinyl)-piperazine, caused increased firing rates only. Gepirone potently inhibited serotonergic impulse flow recorded from the dorsal raphe nucleus (88.3% after 0.04 mg/kg) and this effect was partially reversed by serotonergic antagonists. Both buspirone and gepirone displaced [^{3}H]-5HT from the $5HT_{1a}$ binding site in the hippocampus with IC_{50} values of 10 and 58 nM, respectively. Non-alkyl substituted aryl-piperazines displaced [^{3}H]-5HT from both $5HT_{1a}$ and $5HT_{1b}$ binding sites. Thus, although gepirone may be a weak postsynaptic 5HT agonist, its primary effect is to decrease 5HT neurotransmission. In support of this conclusion was the observed potentiation of antiaggressive effects by blocking 5HT receptors wit small doses of methiothepin or methysergide, which would exacerbate the decreased release of 5HT caused by gepirone. These results are in harmony with reports that decreased serotonergic activity has anxiolytic-like effects in animal models of anxiety.

Key words: Gepirone – Buspirone – 5-Hydroxytryptamine – Receptors – Anxiety

Introduction

Buspirone is a non-benzodiazepine drug that is equipotent to diazepam for relief of anxiety (Goldberg and Finnerty 1979; Rickels et al. 1982). However, buspirone shows neither affinity for nor interactions with the benzodiazepine-GABA-chloride ionophore complex either in vitro (Riblet et al. 1982) or in in vivo (McMillen et al. 1983; Sanghera and German 1983). Thus, buspirone does not exhibit the other pharmacological effects common to the benzodiazepines: i.e. it is not sedating, not a muscle relaxant and does not protect rodents from convulsants (Riblet et al. 1982). Rather than exacerbate the sedation and motor incoordination produced by ethanol, buspirone slightly improves performance by human volunteers (Moskowitz and Smiley 1982). Therefore, buspirone represents a new class of anxiolytic agents.

It was suggested that buspirone may have a dopaminergic action as part of its antianxiety mechanism of action (Riblet et al. 1982). Indeed, buspirone potently increased dopamine metabolism in the striatum (Cimino et al. 1983; McMillen et al. 1983; Wood et al. 1983), but this effect was attributed to strong inhibition of dopamine autoreceptors regulating synthesis on the nerve endings and impulse flow at the cell bodies without substantial inhibition of postsynaptic dopamine receptors (McMillen et al. 1983). In harmony with this conclusion was the observation that the dose-response curve for buspirone elevation of frontal cortical dopamine metabolism, an area lacking autoreceptor control (Bannon et al. 1981), was very flat compared to the striatum (McMillen and McDonald 1983). In addition, McMillen and Mattiace (1983) showed that although gepirone (MJ 13805) could reverse neuroleptic induced catalepsy like buspirone, this analog of buspirone had only minimal effects on dopaminergic neurotransmission. Since buspirone could markedly increase dopamine neuronal impulse flow greater than after typical neuroleptics and both buspirone and gepirone could reverse catalepsy induced either by dopamine receptor blockade or dopamine depletion, McMillen and Mattiace (1983) concluded that buspirone and gepirone were having an effect on the extrapyramidal system efferent from the dopamine receptor in the corpus striatum.

A possible site of action for these two drugs is the serotonergic system. Buspirone inhibits the binding of [^{3}H]-5-hydroxytryptamine to hippocampal membranes (Glaser and Traber 1983) and decreases 5-hydroxytryptamine (5HT) synthesis in rat forebrain (Hjorth and Carlsson 1982). Buspirone potently decreases impulse flow in the serotonergic neurons of the raphe nucleus (VanderMaelen and Wilderman 1984) and serotonergic lesions reduce the effectiveness of both drugs in the conflict (punished reward) test (Eison et al. 1983a, b). It is known that lesions of the ascending serotonergic system can mimic anti-punishment

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effects of benzodiazepines (Tye et al. 1979) and reduce the cataleptic effects of a neuroleptic (Carter and Pycock 1978). However, buspirone has weak efficacy in the conflict test (Weissman et al. 1984) and it is difficult to generate dose-response curves for either drug in this paradigm. In turn this makes comparisons between buspirone's biochemical and behavioral effects difficult and a clear demonstration of buspirone's mechanism(s) of action remains elusive.

The following experiments examined the effects of gepirone (Fig. 1) on brain monoamine neurotransmission in both rats and mice. Gepirone was used primarily throughout the experiments in order to avoid the direct dopaminergic effects of buspirone. Gepirone is known to reduce anxiety scores in limited clinical trials (Cott et al. 1985). Mice were used for isolation-induced aggression as an antianxiety test paradigm (DaVanzo et al. 1966) and rats were used for single cell recordings and receptor binding assays. Dose-response curves in the behavioral tests then could be compared to alterations of dopamine and serotonin metabolite levels, alterations of dopamine and serotonin neuronal unit impulse flow and affinity of gepirone for different receptors. Other serotonergic agonists (substitued aryl-piperazines and 8-hydroxydipropylaminotetralin, Fig. 1) and antagonists were used to help elucidate whether gepirone was producing its behavioral effects by alterations of serotonergic activity. The data demonstrate a close link between the antianxiety (or antiaggression) effect of gepirone and activity at subsets of serotonin receptors.

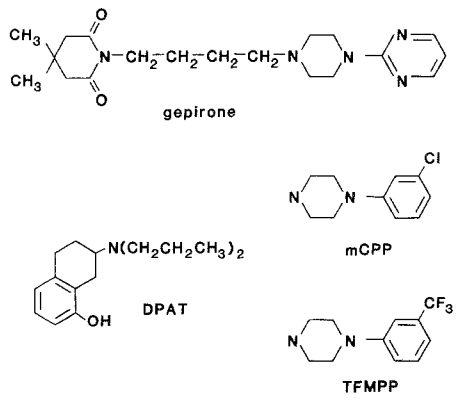


Fig. 1. Structures of gepirone, m-chlorophenylpiperazine (mCPP), m-trifluoromethylphenylpiperazine [also known as 1-(α,α,α-trifluoro-m-tolyl)-piperazine, TFMPP] and (±)-8-hydroxydipropylaminotetralin (DPAT)

Methods

Animals. Female Sprague-Dawley rats, 200–250 g (bred in the Animal Resources Center at E.C.U.) were used for the radioligand binding assays, male Sprague-Dawley rats, 265–385 g (Holtzman, Madison, WI, USA) were used for single cell recordings and male CD-1 mice (Charles River Laboratories, Boston, MA, USA) 20–30 g were used for aggression testing. Rats were killed by decapitation, the brains rapidly removed, rinsed in ice cold saline, blotted and dissected according to Glowinski and Iversen (1966). After removing the corpus striatum, tissue ventral to the anterior commissure was removed and discarded, the septum discarded and the lateral ventricles followed forward to cut out prefrontal cortex (60–80 mg of tissue wet weight) which was trimmed of any olfactory bulbs or tracts. The hippocampus was stripped from the overlying cortex and saved for assay. All of the frontal cortex was saved and used for assay of $5HT_1$ and $5HT_2$ binding sites. Mice were killed by cervical dislocation and a similar dissection procedure followed. Those mice that were placed in individual isolation cages initially weighed 15–20 g at the time of isolation and 30–35 g during aggression training and testing.

Behavioral experiments. For the intraspecies aggression testing, mice were individually housed in 12.5 × 5 cm suspended metal cages for 3 weeks. Then they were trained to attack an intruder mouse (DaVanzo 1969). Training consisted of prodding with long forceps and bumping with an aggregated (group housed) mouse. An aggregated mouse was then left in the cage for 180 s or until a sustained attack occurred. After 5 once daily sessions, over 90% of the mice would attack an intruder in less than 60 s and most in less than 10 s. Only those mice with attack latencies consistently less than 60 s were used for drug experiments. On drug test days, the mice were first tested for fighting, then injected with drug(s) and retested 30 min later. A criterion of 180 s was used for inhibition of attack. Both latency and incidence of attack were recorded.

If sedation or ataxia were produced by any of the various drugs, there could be inhibition of fighting due to these non-specific effects. Thus, each drugs was tested for dose-response effects on the ability of mice to walk a rotating rod (20 r.p.m.; Columbus Instruments) and doses of drugs chosen on the basis of little effect in this test. Mice were trained until they could remain on the rod for 180 s and then used for drug testing. A criterion of 60 s was used to indicate no effect: the shorter time eliminated false results due to gepirone-treated mice jumping off the rod and running away. Furthermore, during the aggression testing, the mice were observed for obvious signs of sedation or ataxia and the experimenter would note whether the mice oriented to or groomed the intruder.

Biochemical assays. A high performance liquid chromatograph was used for assay of dopamine and its metabolites, homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) and 5HT and its metabolite, 5-hydroxyindoleacetic acid (5HIAA). Brain tissue areas were homogenized in 0.4 N $HClO_4$ containing 100 ng of epinine as internal standard. After centrifugation, the supernates were filtered and 20 µl injected into an HPLC system consisting of a Gilson 302 pump, Rheodyne injection port, Brownlee guard column, a Beckman 25 cm 5 µm ODS Ultrasphere column and a Tacussel ED-110 amperometric detector with a glassy carbon electrode. The mobile phase was 0.1 M Na_2HPO_4 and 0.1 M citric acid buffer, pH 4.6, containing 0.25 mM sodium octyl sulfonate and 0.1 mM Na_2EDTA and mixed 88/12 with methanol, then filtered, degassed and pumped at 1.0 ml/min. None of the acute drug treatments altered levels of DA or 5HT in mice brains and only data for the metabolites are reported in the following section.

Binding of [^{3}H]-5HT by prefrontal cortical and hippocampal membranes was used to determine affinity of different drugs for serotonin receptors. The method was a modification of that described by Nelson et al. (1978). Tissues were homogenized in 50 vol. of 50 mM HEPES/KOH pH 7.4 buffer with a Brinkman Polytron (setting 6,

30 s) and centrifuged at 40,000 × g × 10 min. The pellets were resuspended with the Polytron in 50 vol. fresh buffer and incubated for 15 min at 37° C. After another centrifugation step, the pellet was resuspended in fresh buffer containing 10 μM pargyline and incubated for 10 min at 37° to inhibit monoamine oxidase activity. The homogenates were centrifuged and resuspended in 50 vol. of HEPES buffer containing 4.0 mM $CaCl_2$ and 0.01% ascorbic acid. A 0.5 ml aliquot of membrane preparation was added to a 12 × 75 mm borosilicate tube containing 1.5 ml of the final buffer, 8×10^{-10} M [^{3}H]-5HT (20,000 cpm, New England Nuclear, Boston, MA, USA) and various concentrations of test drugs or 10^{-5} M 5HT to determine absolute nonspecific binding. After a 15 min incubation at 37°, the triplicate samples were vacuum filtered through Whatman GF/C filters and the tubes rinsed twice with 2.0 ml of buffer. Radioactivity trapped on the filters was determined by liquid scintillation counting in a Beckman LS9000 scintillation counter (50% efficiency). Linear regression analysis was used to determine the IC_{50} concentrations from semilog plots of the displacement curves. Since some of the drugs displaced only a portion of the serotonin receptor binding (i.e. $5HT_{1a}$ vs. $5HT_{1b}$), the assymptote for that drug was used to calculate a site specific IC_{50}, otherwise no value could be obtained.

In order to determine the affinity of various drugs for D_2 or $5HT_2$ receptors, the ability of drugs to displace [^{3}H]-spiperone from either striatal membranes or frontal cortical membranes was determined (Farber et al. 1983; McMillen 1985). Striatal tissue was used for D_2 receptor binding. Tissues were homogenized in 100 vol. of Na/KPO_4 pH 7.4 buffer (0.05 M Na_2HPO_4 and the pH adjusted with 0.05 M KH_2PO_4), washed twice by centrifugation and a 200 μl aliquot added to 2.8 ml of buffer containing 10^{-10} M [^{3}H]-spiperone (30,000 cpm, Amersham Corp.) and various concentrations of test drugs in 13 × 100 mm borosilicate tubes. The triplicate samples were incubated for 30 min at 37°C and the incubation terminated by vacuum filtration through Whatman GF/C filters, followed by two 3.0 ml rinses with buffer of each tube. Nonspecific binding was determined by adding 2×10^{-7} M (+)-butaclamol to the tubes. Frontal cortical tissue was used for $5HT_2$ receptor binding. Tissues were homogenized in 50 vol. of buffer and washed twice. A 500 μl aliquot was added to 2.5 ml buffer containing [^{3}H]-spiperone and 10^{-5} M (−)-sulpiride (to eliminate D_2 binding) and various concentrations of test drugs or 2×10^{-7} M mianserin to determine nonspecific binding. Incubations were for 30 min at 37° and terminated by vacuum filtration, followed by two 3.0 ml rinses of each tube with buffer and a final 3.0 ml rinse of each sample well. The filters were placed in scintillation vials and mixed with 10 ml of Liquiscint (National Diagnostics Co.) and the radioactivity determined by scintillation counting. The IC_{50} values for each drug were determined from linear regression analysis of semilog plots of the displacement curves.

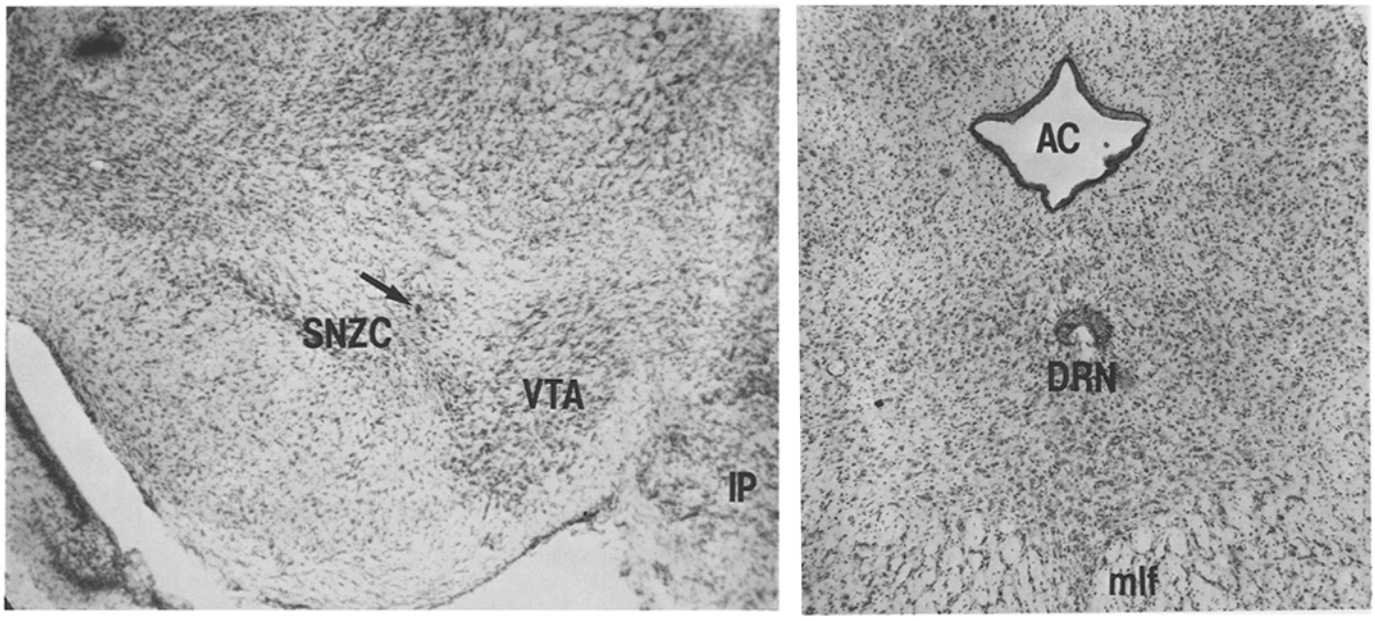
Electrophysiology. Extracellular single cell recordings were made from dopamine (DA) cells located in the substantia nigra zona compacta (SNZC) and from 5HT containing neurons of the dorsal raphe nucleus (DRN). In these experiments, male Sprague-Dawley rats, weighing 265−385 g, were anesthetized with chloral hydrate (400 mg/kg i.p.) and the femoral vein catheterized. All incision sites and pressure points were infiltrated with a long lasting local anesthetic, 2% mepivacaine-HCl. The rats were placed in a stereotaxic apparatus (Kopf Instruments) and body temperature maintained between 36−37° C throughout the experiment. For DA cell recordings, a burr hole was drilled with its center 2.8−3.2 mm anterior from earbar zero and 1.8−2.2 mm lateral from midline suture. For 5HT cell recordings, a burr hole was drilled in the midline with its center 0.5−0.8 mm anterior to lambda. After removal of the dura overlying the brain, a tungsten electrode (2−4 Mohms, Fredrick Haer Co.) or a glass micropipette filled with fast green dye was hydraulically lowered into the DA or 5HT cell regions using coordinates from the atlas of Konig and Klippel (1963).

Extracellular action potentials were passed through a high input impedance amplifier (Grass P511, 0.3−3.0 kHz bandpass) and monitored on an oscilloscope and audio amplifier. A Schmitt trigger was set such that its output was triggered by a single action potential and the output was counted, displayed and stored in histogram form (MICRO LSI 11/23) computer, bin width 10 s). Baseline neuronal activity was taken for at least 20 bins prior to any drug administration. Only one cell per animal was studied pharmacologically and the drug responses were measured as percentage changes from baseline firing levels. At the end of each experiment, the anatomical location of the recorded cell was determined by passing +10 μA current for 10 s through the tungsten electrode or by passing −20 μA current through the glass recording micropipette for 30 min. Animals were then overdosed with pentobarbital and perfused transcardially with neutral buffered formalin. The brain was sectioned on a freezing microtome at 50 μm, the sections stained with cresyl violet and the recording sites were histologically verified. Examples of recording sites are shown in Fig. 2.

Although the identity of DA and 5HT neuronal recording sites were determined retrospectively by histological examination, certain electrophysiological characteristics of DA and 5HT neurons were used to identify these cells in the SNZC and the DRN. DA cells were identified by their long duration action potentials (> 2 ms) and slow discharge rates (1−9 impulses/s). These cells often fired in bursts with successive spikes within each burst exhibiting decreased spike amplitudes (Bunney et al. 1973; German et al. 1979). The 5HT cells in the DRN were recognized as positive-negative going spikes with rhythmic firing patterns and slow rates (0.5−3.0 impulses/s) appearing in the midline just ventral to an area of electrical quiescence, the cerebral aqueduct (Wang and Aghajanian 1977; Trulson et al. 1982).

To determine the effect of chloral hydrate anesthesia on neuronal drug responses, a small group ($n = 4$) of animals were prepared in accordance with the guidelines of the American Physiological Society. These rats, while under halothane anesthesia, were tracheotomized, the femoral veins catheterized and placed in a stereotaxic apparatus for dopaminergic recording as previously described. The animals were then discontinued from halothane, paralyzed with gallamine triethiodide (16 mg/kg) via the femoral vein. All animals were then respired on room air using a Harvard Inst. respirator. At least 40 min were allowed for halothane to be completely removed before any data were collected.

Statistics. For multiple comparisons to a control, a one-way ANOVA followed by Dunnett's t test was used (Zar 1984). Where appropriate a Student's t test was used for



a b

Fig. 2a, b. Histological verification of DA and 5HT cell recording sites. In **a** the arrow points to a cell stained with fast green dye located in the substantia nigra zone compacta. In **b** a microlesion is located just above the abbreviation DRN which is in the dorsal raphe nucleus. Abbreviation: *AC* cerebral aqueduct; *DRN* dorsal raphe nucleus; *IP* interpeduncular nucleus; *mlf* medial longitudinal fasciculus; *SNZC* substantia nigra zona compacta; *VTA* ventral tegmental area

comparisons between two independent groups. Analyses were run on an Apple IIe microcomputer using programs by Barlow (1983). A two tailed value of P at least less than 0.05 was taken to indicate a significant difference. The ED_{50} for gepirone inhibition of mouse aggression was calculated according to Litchfield and Wilcoxon (1949).

Drugs and sources. The following drugs were generously donated: buspirone-HCl, gepirone-HCl, 1-(2-pyrimidinyl)-piperazine-HCl (1PP) and m-chlorophenyl-piperazine-HCl (mCPP, Bristol-Myers Co., Evansville, IN, USA), methysergide-maleate (Sandoz Pharmaceuticals, East Hanover, NJ, USA), methiothepin-maleate (Hoffmann-LaRoche Inc., Nutley, NJ, USA), (+)-butaclamol-HCl (Ayerst Laboratories, Montreal, Canada), SKF 525A (proadifen-HCl, Smith Kline & French Labs, Philadelphia, PA, USA), haloperidol (Janssen Pharmaceutica, Beerse, Belgium), mianserin-HCl (Organon, Oss, The Netherlands), (−)-sulpiride (Ravizza S.P.A., Milano, Italy). The following drugs were purchased: (±)-8-hydroxydipropylamino-tetralin-HBr (DPAT, Research Biochemicals Inc., Wayland, MA, USA), 1-(α,α,α-trifluoro-m-tolyl)-piperazine-HCl (TFMPP, Aldrich Chemical Co., Milwaukee, WI, USA) and pargyline-HCl (Sigma Chemical Co., St. Louis, MO, USA). All doses refer to the free base form of drug.

Results

1. Inhibition of mouse aggression. Both gepirone and mCPP could inhibit intraspecies aggression by mice. Figure 2 shows that 10 mg/kg i.p. of either drug produced near 100% inhibition of fighting in these isolated mice. Thus, gepirone is about twice as potent as mCPP on a molar basis. The calculated ED_{50} value for gepirone was 4.5 mg/kg (95% confidence limits: 2.8 − 7.0 mg/kg). Buspirone had an ED_{50} of 3.5 mg/kg in this test paradigm (McMillen, 1986). Another simple aryl-piperazine, TFMPP, had a dose-response curve similar to mCPP: 10 mg/kg i.p. inhibited fighting by 7 of 8 mice (Table 1). In resident male intruder models, benzodiazepines often show biphasic dose-response curves: low doses actually decrease attack latency (Miczek 1974). However, with these trained mice, the average pre-drug attack latency was less than 5 s which makes determining a biphasic effect impossible.

Table 1. Effects of TFMPP or DPAT on isolation-induced agressive mice

Drug	Fighting/tested	
TFMPP		
1.25	8/8	
2.5	8/8	
5.0	1/8	
10.0	0/8	
DPAT		+2.5 methyser.
0.25	8/8	2/8
0.5	5/8	3/8
1.0	2/8	0/8
2.0	0/8	0/8

Isolated mice were tested for fighting, drug injected (doses are mg/kg i.p.) and retested 30 min later. A criterion of 180 s for attack on a group-housed intruder was used to determine inhibition of fighting behavior. An additional group of isolated mice received both various doses of DPAT and 2.5 mg/kg i.p. methysergide

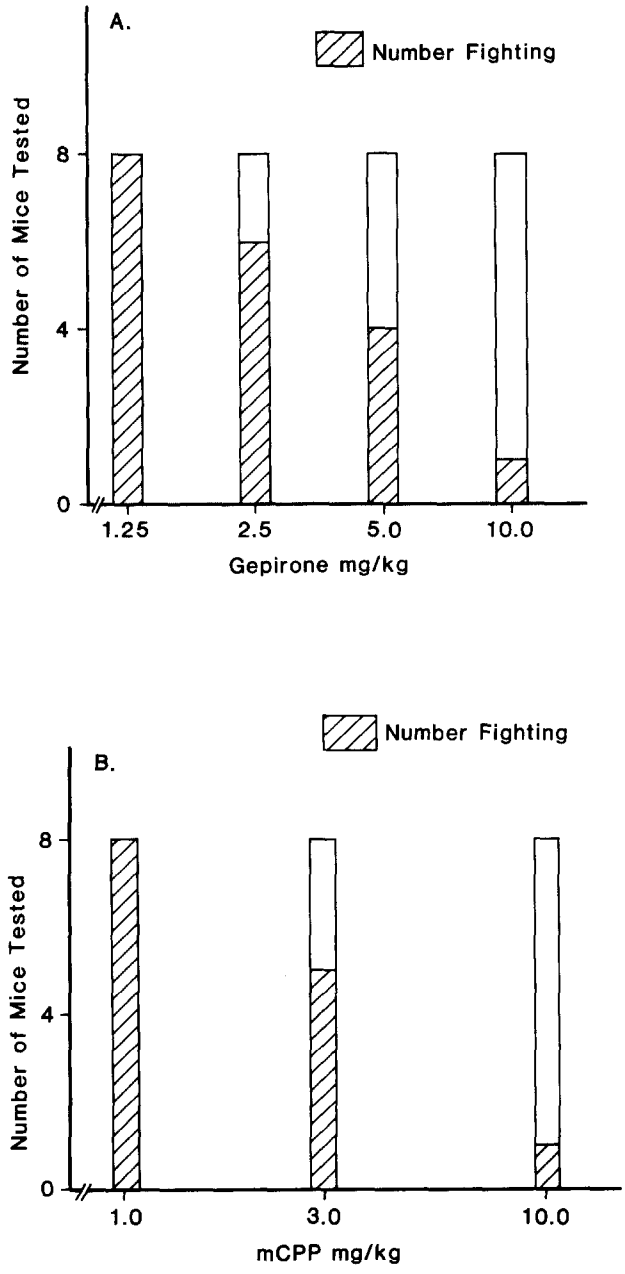


Fig. 3. Effect of gepirone (**A**) or mCPP (**B**) on fighting by isolated aggressive mice. Isolated mice were tested for fighting, drug injected i.p. and retested 30 min later. A criterion of 180 s for attack on a group housed intruder was used to determine inhibition of fighting behavior. Bars are spaced on a log scale

The dose-response curve for gepirone was repeated with coadministration of 0.25 mg/kg i.p. methiothepin. Figure 3 shows that methiothepin potentiated the effects of gepirone. For example, 5.0 mg/kg of gepirone alone inhibited fighting in 50% of the mice tested (Fig. 1), but inhibited 100% of the mice tested which were treated with methiothepin ($p < 0.01$, χ^2). Coadministration of 2.5 mg/kg i.p. methysergide resulted in 100% inhibition of fighting after the 5.0 mg/kg dose of gepirone (data not shown). These doses of serotonin antagonists did not inhibit aggression when administered alone. Thus, inhibition of 5HT receptors potentiated the antiaggressive effect of gepirone. In contrast, coadministration of either methiothepin or methysergide did not alter the inhibition curve produced by mCPP. These two antagonists were chosen because their non-5HT effects are different from each other. In addition, the potent direct acting $5HT_{1a}$ agonist, DPAT (Hjorth et al. 1982), was tested in this aggression paradigm. There was no inhibition and no increase in latency to attack with doses of up to 0.5 mg/kg i.p. and 75% inhibition of attack after 1.0 mg/kg (Table 1). Also, shown in Table 1 is that 2.5 mg/kg of methysergide potentiated the inhibition of aggression by DPAT. Although rats adminstered doses in the 0.5 to 2.0 mg/kg range of this drug normally exhibit a 'serotonin syndrome' (Hjorth et al. 1982), these mice did not: either they are less sensitive to this effect of DPAT or they have a greater rate of metabolism of the drug than rats.

Table 2. Effects of various drugs on the ability of mice to stay on a rotating rod

Drug mg/kg i.p.	Seconds ±SEM
H_2O or vehicle	60.0 ± 0.0 (10)
Gepirone	
5.0	60.0 ± 0.0 (5)
10.0	46.6 ± 8.3 (5)
mCPP	
5.0	37.2 ± 7.9 (5)
10.0	26.0 ± 9.3 (5)
DPAT	
1.0	60.0 ± 0.0 (5)
Methysergide	
2.5	60.0 ± 0.0 (5)
+5.0 gepirone	52.2 ± 7.8 (5)
+5.0 mCPP	60.0 ± 0.0 (5)
+1.0 DPAT	60.0 ± 0.0 (5)
Methiothepin	
0.25	60.0 ± 0.0 (5)
+5.0 gepirone	35.8 ± 8.5 (5)
+5.0 mCPP	12.6 ± 4.2 (5)
+1.0 DPAT	35.8 ± 9.9 (4)

Mice were trained to stay on a rotating rod for 180 s. On the test day, the mice were first tested on the rod, then drugs were injected in the doses shown and the mice retested 30 min later. A time of 60 s was taken as indicating little effect on locomotor ability. Numbers in parentheses represent the number of mice in each group

Table 2 shows some of the data from the rotorod testing. The mice tolerated gepirone and DPAT very well. Both mCPP and TFMPP were much more disruptive of motor ability (data for TFMPP is similar to mCPP) although even when combined with methiothepin the mice would still orient to and attack intruders. Methiothepin was the most disruptive drug, which may reflect its strong affinity for the D2 receptor binding site. Methysergide, alone or in combination with other drugs, was well tolerated with little effect on rotorod performance and, although gepirone and DPAT were potentiated in the aggression test, these mice were mobile and oriented to the intruders.

An import question with regards to the buspirone type drugs is whether the rapid metabolism of these drugs is necessary for activity or are the parent drugs active themselves? Buspirone is rapidly metabolized to 1-PP and several hydroxylated metabolites (Caccia et al. 1982; Gammans et al. 1983) and its ability to increase dopamine

Table 3. Alterations of dopamine metabolism by gepirone in aggregated male mice

		A. HVA μg/g ± SEM	
		Striatum	Prefrontal cortex
Control gepirone	(9)	2.15 ± 0.12	0.32 ± 0.06
0.5	(7)	1.98 ± 0.22	0.25 ± 0.03
1.25	(7)	1.70 ± 0.07**	0.21 ± 0.01
2.5	(6)	1.75 ± 0.20**	0.23 ± 0.04
5.0	(6)	2.66 ± 0.31*	0.38 ± 0.06
$F_{4,29}$		3.585 $p < 0.05$	2.063 N.S.
		B. DOPAC μg/g ± SE	
		Striatum	Frontal cortex
Control gepirone	(9)	1.46 ± 0.08	0.14 ± 0.02
0.5	(7)	1.15 ± 0.08	0.12 ± 0.02
1.25	(6)	1.21 ± 0.16	0.12 ± 0.02
2.5	(6)	1.82 ± 0.31**	0.13 ± 0.02
5.0	(6)	2.17 ± 0.26**	0.15 ± 0.02
$F_{4,29}$		5.241 $p < 0.01$	

Different from control: $*p < 0.05$, $**p < 0.01$ (Dunnett's t-test). Various doses of gepirone (mg/kg) were injected i.p. into mice and the animals killed 30 min later. Numbers in parentheses represent number of mice in each group

Table 4. Alterations of serotonin metabolism by gepirone in aggregated male mice

		5HIAA μg/g ± SEM	
		Striatum	Prefrontal cortex
Control gepirone	(9)	0.532 ± 0.029	0.326 ± 0.017
0.5	(7)	0.564 ± 0.044	0.321 ± 0.033
1.25	(6)	0.466 ± 0.057	0.241 ± 0.026
2.5	(6)	0.362 ± 0.036**	0.225 ± 0.037
5.0	(6)	0.447 ± 0.055*	0.258 ± 0.024
$F_{4,29}$		3.990 $p < 0.05$	3.076 N.S.

Different from control: $*p < 0.05$, $**p < 0.01$ (Dunnett's t-test). Various doses of gepirone (mg/kg) were injected i.p. into male mice and the animals killed 30 min later. Numbers in parentheses represent number of mice in each group

metabolism shows a first pass effect (McMillen and McDonald 1983). Gepirone undergoes similar metabolic processes and very little drug is detectable after p.o. dosage (Garattini, personal communicaton). However, 10 mg/kg i.p. of 1-PP inhibited aggression in 4 of 8 mice tested. An additional 6 mice were pretreated with 20 mg/kg i.p. SKF 525A (a dose without effect on incidence or latency for fighting) 5 min before 5.0 mg/kg i.p. gepirone. Attack was inhibited in all of these mice: i.e. inhibition of liver microsomal drug oxidation had potentiated the effectiveness of gepirone. Thus, gepirone itself is an effective anti-aggressive drug.

2. Effects of gepirone on monoamine metabolism. As previously reported for rats (McMillen and Mattiace 1983), gepirone had modest effects on dopamine metabolism in mouse striatum and frontal cortex. Significant changes occurred only in the striatum. As the dose of gepirone was increased from 0.5 to 5.0 mg/kg i.p., DOPAC concentrations increased and reached signfificance after the largest dose (Table 3). In contrast, stritial HVA concentrations initially declined with increasing doses of gepirone, but after the 5.0 mg/kg dose HVA concentrations became elevated (Table 3). The declining HVA levels combined with increasing DOPAC levels suggested that gepirone increased intraneuronal metabolism of dopamine. Apparently, after a large enough dose of gepirone, both HVA and DOPAC concentrations increased, which suggested that dopamine metabolism was increased. These variable effects were in harmony with the variable effects of gepirone on dopaminergic neuronal impulse flow described below.

Gepirone decreased serotonin metabolism in both areas examined. About a 1/3 decrease was the maximal effect after a dose of 2.5 mg/kg i.p.: the 5.0 mg/kg dose produced less of an effect than the 2.5 mg/kg dose (Table 4). Thus, these data are similar to that reported for the effects of buspirone on the rat by Hjorth and Carlsson (1982). The data indicate that the behavioral effects began to occure as the decrease in serotonin metabolism reached maximum.

3. Effects on single cell impulse flow. The dopaminergic responses to gepirone in the chloral hydrate anesthetized rats were found to be similar in dosage, intensity and direction to those obtained in the gallamine paralyzed group. Data obtained from both groups are therefore compiled together and the results are discussed without any distinction being made to the type of anesthesia used.

The effect of gepirone on dopamine neuronal impulse flow was found to be very different from that previously reported for buspirone both in dosage and direction of the responses (McMillen et al. 1983). Whereas the predominant action of buspirone of dopamine cells was one of excitation with low doses (0.2 – 2.0 mg/kg), the effect of gepirone on dopaminergic impulse flow was that of inhibition at high doses. Of the six cells tested for their response to i.v. gepirone, four had their firing rates reduced by 20 – 40% with doses of 2.3 – 10 mg/kg (Fig. 5A). One nigral cell showed an increase of 30% in firing rate after 10 mg/kg of gepirone and the remaining cell was unaffected with doses up to 20 mg/kg. In each cell the first dose produced the most dramatic and sustained change in baseline firing with subsequent doses having little further effect. The direct dopamine agonist, apomorphine (APO), when administered after gepirone decreased nigral impulse flow only at high doses ($ID_{50} = 60$ μg/kg; $n = 5$).

In a second set of experiments the ability of gepirone to reverse the apomorphine induced inhibition of dopaminergic impulse flow was tested. In all cells tested ($n = 4$), apomorphine signficantly inhibited impulse flow ($ID_{50} =$ 8.5 μg/kg) and gepirone increased the firing rate to or towards baseline levels in every case (Fig. 5B). However, gepirone was not as potent as buspirone for reversal of the apomorphine induced inhibition and never raised firing rates above baseline as does buspirone. In those cells where the firing rate had not returned to baseline levels with gepirone, i.v. administration of the dopamine receptor antagonist haloperidol (0.2 mg/kg) increased nigral impulse flow further to above baseline levels (Fig. 5A).

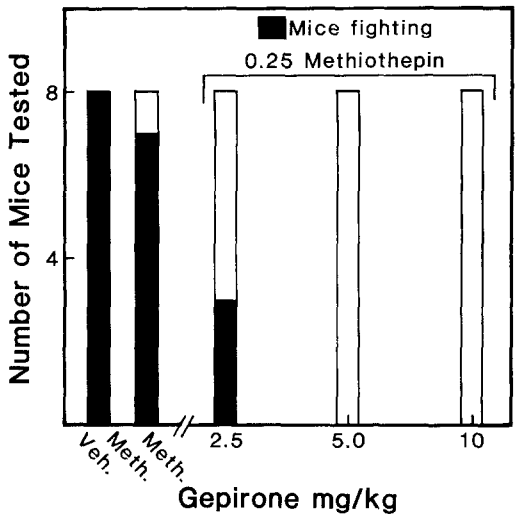


Fig. 4. Effect of combining methiothepin with various doses of gepirone on fighting by isolated aggressive mice. Isolated mice were tested for fighting, drug(s) or vehicle injected and retested 30 min later. A criterion of 180 s for attack on a group housed intruder was used to determine inhibition of fighting. Methiothepin significantly increased the effectiveness of the 5.0 mg/kg dose of gepirone ($p < 0.01$, Chi square). Bars are spaced on a log scale

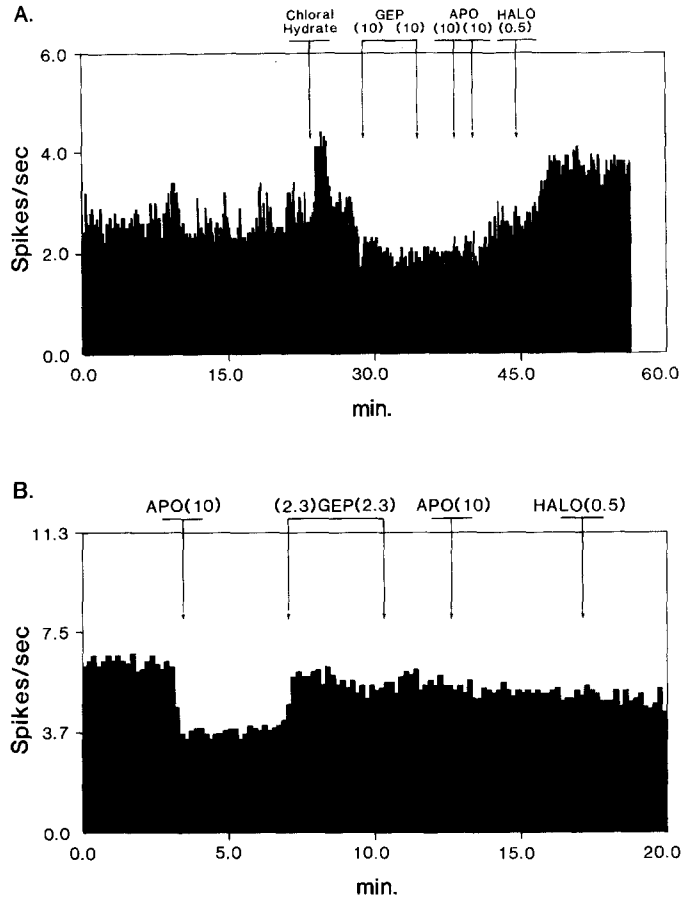


Fig. 5A, B. Effect of gepirone (*GEP*) on substantia nigra zona compacta cells. **A** 10 mg/kg i.v. gepirone decreased the firing rate (averaged every 10 s) 32% with no further effect by either a second dose or 20 μg/kg i.v. apomorphine (*APO*). Haloperidol (*HALO*, 0.5 mg/kg i.v.) increased firing rate to slightly greater than control. **B** Apomorphine (10 μg/kg i.v.) was administered first and caused a 41% decrease in firing rate which was reversed by the first dose of gepirone. In this recording, haloperidol had no further effect

The common metabolite of buspirone and gepirone, 1-PP, was tested on 8 dopamine cells. Cumulative doses of 0.8 to 3.2 mg/kg i.v. 1-PP had little effect on dopaminergic activity in all cells tested ($n = 4$). However, with single doses of 1.0 or 5.0 mg/kg, 1-PP produced a sustained increase in the firing rate above baseline levels (50–70%) in another four cells. An additional dose of 10 mg/kg did not further increase the rate, but changed the firing pattern dramatically into one of decremental bursting ($n = 4$) to the point that three of the cells underwent depolarization block that could be reversed by administration of apomorphine (10–20 μg/kg). Pretreatment with 5.0–7.5 μg/kg apomorphine to lower baseline firing rates prevented the depolarization block phenomenon ($n = 3$). A total dose of 16 mg/kg 1-PP did not alter firing rate or pattern after apomorphine-induced inhibition of impulse flow, but haloperidol (0.03 mg/kg) increased firing rates in this recording.

The serotonin containing cells of the dorsal raphe nucleus were extremely sensitive to gepirone administration. The first two rats studied had 0.1 or 0.05 mg/kg gepirone injected and impulse flow was inhibited 100% in both cases without recovery during the course of the experiment. Those animals receiving low doses of gepirone, exhibited only partial reversals of serotonergic impulse flow after administration of the serotonergic antagonists, methiothepin or methysergide (Fig. 6A and B). All seven cells tested with gepirone were inhibited by doses of gepirone 100-fold less than used in the substantia nigra recordings. Figure 6A and B show that doses of 0.005 mg/kg i.v. gepirone briefly decreased raphe cell firing rates. Cumulative doses of 0.04 mg/kg or less decreased serotonergic impulse flow by $88.3 \pm 5.2\%$ ($n = 4$). Only partial reversal of gepirone induced inhibition of serotonergic impulse flow was obtained with the antagonists, methiothepin or methysergide, in 3 of 5 cells tested (Fig. 6A and B). In the remaining two cells neither of these drugs altered the gepirone induced inhibition of 5HT impulse flow. The simpler aryl-piperazine, mCPP, had less of an effect on impulse flow than gepirone. Doses of 0.05–0.1 mg/kg decreased impulse flow by 25–65% ($n = 3$; Fig. 6C).

4. Affinity for dopamine and serotonin receptors. The majority of drugs had little affinity ($IC_{50} > 10^{-6}$ M) for the D_2 binding site on striatal membranes, with the exception of methiothepin. However, some of these drugs could displace [^{3}H]-spiperone binding at concentrations up in the 1–10 μM range (Table 5), which indicated that dopaminergic effects could occur if doses were very large. The displacement curve for gepirone was very flat and indicated little affinity for the striatal D_2 binding site.

Of the agonists tested, DPAT had the highest affinity for 5HT receptor sites (Table 5; Fig. 7). Gepirone, buspirone and DPAT could displace over two thirds of specific [^{3}H]-5HT binding to hippocampal membranes, but only 40% of the binding in frontal cortex. These results are consistent with previous reports that $5HT_{1a}$ binding sites are concentrated in the hippocampus (Maracinkiewicz et al. 1984; Pazos and Palacios 1985) and that [^{3}H]-buspirone concentrates in the hippocampus after i.v. administration (Kaulen et al. 1985; Taylor et al. 1984). In contrast, mCPP

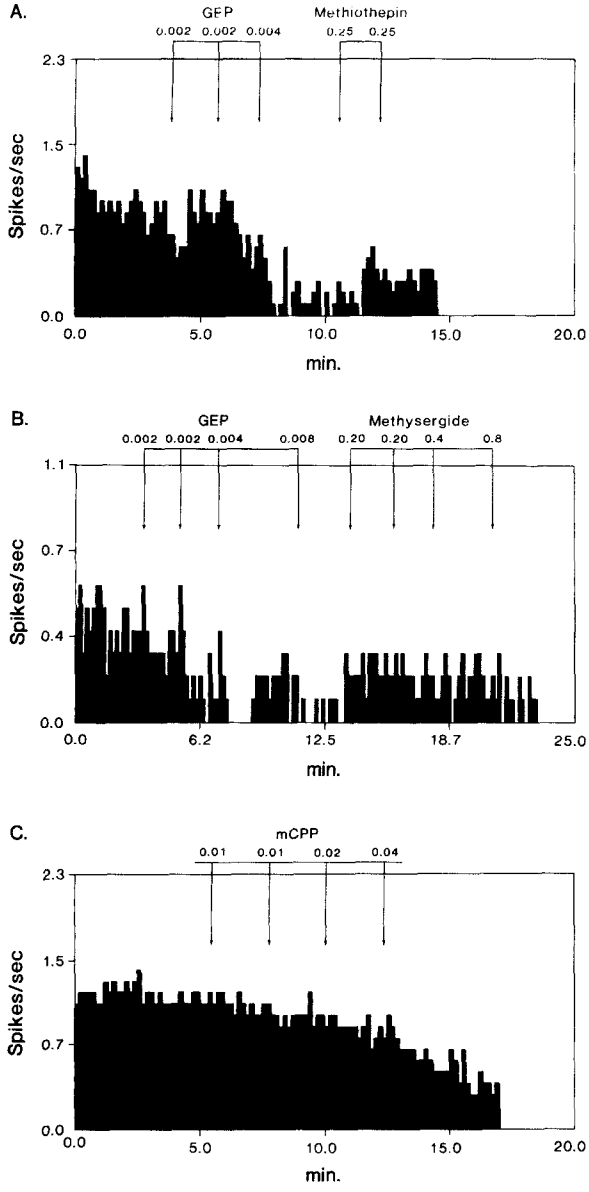


Fig. 6A–C. Effect of gepirone (*GEP*) or mCPP on dorsal raphe nucleus cells. **A** A total dose of 0.008 mg/kg i.v. gepirone inhibited impulse flow 85%. Methiothepin (0.5 mg/kg i.v. total dose) partially reversed this effect. **B** Another cell potently inhibited by gepirone (0.016 mg/kg i.v. total dose) which was partially reversed by the first dose (0.2 mg/kg i.v.) of methysergide. **C** Inhibition of impulse flow by mCPP (0.08 mg/kg i.v.) reached 65% before the cell was lost

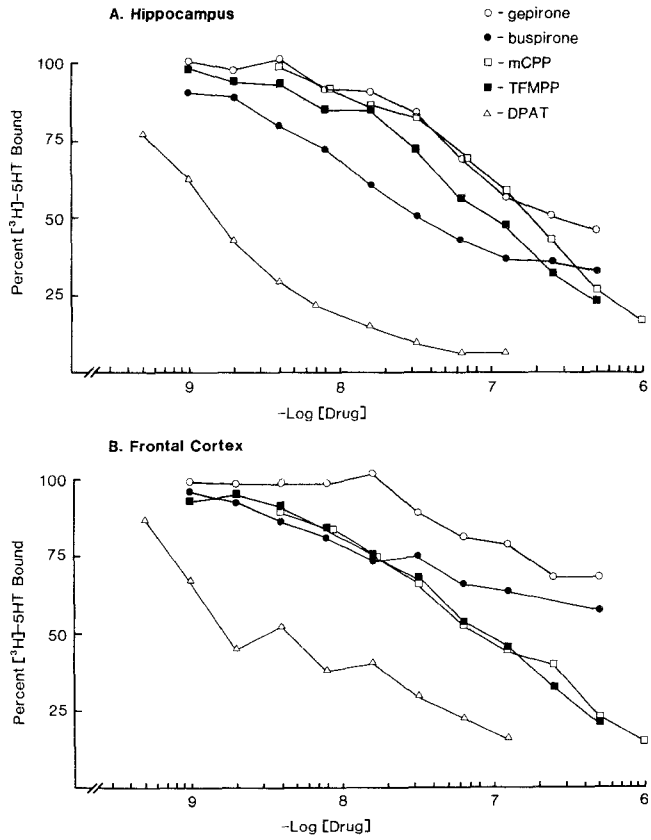


Fig. 7. Ability of various drugs to displace [^{3}H]-5HT from either hippocampal (**A**) or frontal cortical (**B**) membranes. Results are reported as % of specific 5HT binding (defined by 10 μM 5HT) and are averaged from 3–4 independent assays for each drug

Table 5. Ability of serotonergic drugs to displace ligands for serotonin or dopamine receptors

Drug	IC_{50} nM ± SEM			
	$5HT_1$ f. cortex	$5HT_1$ hippo.	$5HT_2$ f. cortex	D_2 striatum
Gepirone	69 ± 7	58 ± 8	5,875 ± 968	2,985 ± 1,681
Buspirone	11 ± 2	10 ± 1	2,015 ± 438	264 ± 12
MCPP	89 ± 22	177 ± 27	606 ± 190	$>10^{-5}$ M
TFMPP	84 ± 9	100 ± 17	781 ± 32	6,345 ± 975
DPAT	3 ± 1.0	1 ± 0.2	$>10^{-5}$ M	$>10^{-5}$ M
Methysergide	24 ± 8	30 ± 3	8.4 ± 1.4	1,079 ± 272
Methiothepin	6 ± 1	5 ± 1	0.13 ± 0.02	1.0 ± 0.4

Each IC_{50} value is the mean of 3–4 separate assays. The $5HT_1$ site was labeled with 8×10^{-10} M [^{3}H]-5HT, the $5HT_2$ site with 10^{-10} M [^{3}H]-spiperone in the presence of 10^{-5} M (–)-sulpiride and the D_2 site with 10^{-10}M [^{3}H]-spiperone with 2×10^{-7} M (+)butaclamol to define specific binding. Since gepirone, buspirone and DPAT displaced a portion of specific [^{3}H]-5HT binding, their saturation assymptotes were used to calculate a site specific IC_{50} value ($5HT_{1a}$ site)

and TFMPP displaced about the same percentage of [^{3}H]-5HT from membranes prepared from both brain areas which is consistent with previous reports that these drugs can displace from both $5HT_{1a}$ and $5HT_{1b}$ binding sites (Peroutka 1985). As ten different concentrations of these drugs were used to determine IC_{50} values, the displacement curve did not allow for discrimination between the two 5HT binding sites by these two drugs. Methiothepin and methysergide could displace all of the specific serotonin binding as would be expected for nonspecific serotonin antagonists. As a further demonstration of selectivity for $5HT_{1a}$ binding sites by buspirone and gepirone, saturating concentrations of these drugs were added to a saturating concentration of DPAT at frontal cortical membrane 5HT binding sites. For comparison, less than saturating concentrations of mCPP and TFMPP were added to the same concentration of DPAT. Table 6 shows that neither gepirone nor buspirone could add to the displacement of [^{3}H]-5HT by DPAT in the frontal cortex. However, mCPP and TFMPP significantly increased the displacement observed with DPAT alone in

Table 6. Additive effects of aryl-piperazines to (±)8-OH-DPAT displacement of [^{3}H]-5HT binding to frontal cortical membranes

Drug (molar)	Percent specific [^{3}H]-5HT bound ± SEM	
	Alone	+DPAT
Control	–	45.8 ± 1.9
Gepirone (5×10^{-7})	40.4 ± 2.0	51.0 ± 3.5
Buspirone (2.5×10^{-7})	46.2 ± 2.0	49.6 ± 3.5
TFMPP (2.5×10^{-7})	67.8 ± 4.0	80.3 ± 3.0*
MCPP (2.5×10^{-7})	54.1 ± 2.9	76.9 ± 2.1*

Different from DPAT alone: * $p < 0.01$ (Dunnett's t test).
Frontal cortical membranes from rats were incubated with 8×10^{-10} M [^{3}H]-5HT either alone or with test drugs in concentrations shown. Additional samples had 2.5×10^{-8} M DPAT added to the incubation media

frontal cortex as these drugs were also binding to the $5HT_{1b}$ site. These data indicate that displacement of [^{3}H]-5HT by DPAT, buspirone and gepirone was occurring at a single site: the $5HT_{1a}$ binding site.

Discussion

These data confirm a previous report that gepirone (MJ 13805) neither inhibits dopamine autoreceptors nor increases dopamine synthesis and metabolism in a manner similar to buspirone (McMillen et al. 1983; McMillen and Mattiace 1983). Gepirone decreased the firing rate of most rat nigral dopamine cells recorded and had mixed effects on dopamine metabolite (DOPAC and HVA) concentrations in mouse brain. McMillen and Mattiace (1983) had concluded that the antianxiety and anticataleptic effects of buspirone and gepirone were mediated outside the dopaminergic system and the present data strengthen that concept. For example, the majority of substantia nigra neurons had reduced firing rates after gepirone, but apomorphine had no further effect. Also, if firing rates were first reduced by apomorphine, gepirone brought the impulse flow back towards baseline. Thus, gepirone was having an effect that seemed to negate the activity of apomorphine on these neurons. This effect resembles partial agonist activity. However, since the doses required were large and gepirone could produce full reversal of apomorphine in some recordings, a non-dopaminergic effect seems the most likely explanation of these data.

Attempts to determine the antianxiety mechanism for these drugs has been hampered by the lack of an animal model for anxiety which was sensitive to buspirone and its analogs. Recently, Witkin and Barrett (1986) described buspirone as having strong effects in the conflict paradigm (punished reward) if pigeons were used instead of rats or monkeys. Olivier et al. (1984) has described buspirone as having antiaggressive effects in mated male rats presented with an intruder in their home cage. However, there also occurred decreased exploratory behavior and the antiaggressive affect was attributed to non-specific sedation. We have noted decreased exploratory behavior following injections of buspirone (McMillen et al. 1983), but this was not accompanied by sedation: i.e. the rats were still alert and responsive to handling.

Isolation-induced aggression between mice is an intraspecies model of aggression that can be inhibited by benzodiazepines, neuroleptics and anticholinergics (DaVanzo et al. 1966). At reasonable doses, neither buspirone nor gepirone will interact with the receptors for these different chemical classes of drugs, yet buspirone potently inhibits attacks by isolated mice against intruder mice (McMillen 1986). Thus, this model appears to be useful for study of sites of activity related to inhibition of aggression (anxiolytic sites?). How either inhibition of aggression or increased punished responding neurochemically relates to anxiolytic activity in humans is unclear. In harmony with studies using the conflict model, gepirone exhibits a potency in the aggression model similar to buspirone: ED_{50} values of 3.5 and 4.5 mg/kg i.p. for buspirone and gepirone, respectively. These doses of gepirone have little effect on the ability of mice to stay on a rotating rod for 60 s, which indicates that the antiaggressive effect is not due to sedative or ataxic effects. The mice are able to orient to the intruder, but do not show aggressive grooming or attack. Therefore, the inhibition of aggression occurred without nonspecific sedative effects and without interactions with dopamine, benzodiazepine or cholinergic receptors. Chlordiazepoxide is known to increase aggression, shorten latencies, in small does then inhibit in larger doses (Miczek 1974). Because the mice used for this study had short latencies for attack to begin with, it was not possible to see potentiation of aggression by small doses of drugs.

The 5HT agonist, mCPP, inhibited aggression with about the same mg potency or about half the molar potency of gepirone. Another simple aryl-piperazine, TFMPP, inhibited aggression, but these two drugs were more disruptive of motor behavior (rotorod) than gepirone although the mice were not obviously sedated. These data were suggestive that gepirone may be acting at the same sites as these halogenated aryl-piperazines: $5HT_1$ binding sites.

However, the receptor binding data show some clear differences between these drugs. First, the ability of gepirone to displace [^{3}H]-5HT from hippocampal membranes is similar to either mCPP or TFMPP, but its ability to inhibit aggression is twice that of these drugs. Secondly, gepirone is clearly displacing from the $5HT_{1a}$ binding site only, while mCPP and TFMPP displace from both $5HT_{1a}$ and $5HT_{1b}$ sites. These results are similar to those described for buspirone by Peroutka et al. (1985). Third, compared to its affinity for $5HT_{1a}$ receptors and its ability to produce the serotonin syndrome, DPAT is as strong an inhibitor of aggression as expected. Yocca et al. (1985) have reported that buspirone and gepirone are partial agonists at the serotonin-stimulated adenylate cyclase receptor in guinea pig hippocampus. Gepirone had an $EC_{50} = 200$ nM and intrinsic activity of 0.6. Andrade and Nicoll (1985) reported that 5HT, buspirone or gepirone hyperpolarized rat CA1 hippocampal pyramidal cells and that the latter two drugs were partial agonists. Thus, post-synaptic agonist effects of these drugs will be muted by their lack of full efficacy.

One possible difficulty in comparing receptor binding data with behavioral data is that gepirone is rapidly metabolized and a more active drug may be formed, which would be absent in the various in vitro assays employed or after i.v. administration. A major metabolite fo both buspirone and gepirone is 1-PP, but this drug lacked comparable efficacy in the aggression test and probably does not account for the behavioral effects of these drugs. In order to test

whether gepirone was undergoing first-pass metabolism to an active metabolite, mice were pretreated with SKF 525-A to inhibit the mixed function oxidase system of the liver. The result was a dramatic increase in the efficacy of gepirone, which indicates that parent drug was an effective antiaggressive drug and that the use of gepirone in binding assays or administration of drug i.v. is legitimate.

Gespirone was tested for its presynaptic effects on dopaminergic and serotonergic impulse flow. Recordings from the substantia nigra showed that the majority of cells were inhibited by gepirone administration and excited by injection of its metabolite, 1-PP. Note that effective doses of gepirone were very large (up to 10 mg/kg i.v.) and may be producing non-specific effects. This is in contrast to buspirone, which inhibited dopamine iontophoresed onto these neurons and consistently increased impulse flow greater than neuroleptics when administered at 0.1 – 1.0 mg/kg systemically (McMillen et al. 1983). McMillen and Mattiace (1983) had reported that gepirone would not block dopamine nerve ending receptors regulating tyrosine hydroxylase activity in marked contrast to buspirone and had therefore dismissed the dopaminergic effects of buspirone as a mechanism for reduction of anxiety. However, buspirone was reported to decrease serotonergic impulse flow (VanderMaelen and Wilderman 1984). In the present study, small doses of gepirone were able to inhibit firing of 5HT containing neurons in the dorsal raphe nucleus. Gehlbach and VanderMaelen (1985) reported similar results with gepirone when recording from serotonergic neurons in rat brain slices. Serotonergic antagonists were able to partially restore firing rates, as is typical for these neurons (Aghajanian and Wang 1978; Rebec et al. 1982; c.f. DeMontigny et al. 1985). Therefore, injection of buspirone or gepirone would appear to cause a decreased release of 5HT, as noted by decreased levels of 5HIAA, while weakly stimulating $5HT_{1a}$ receptors in hippocampus.

In order to determine which site was important for drug action, serotonin antagonists, methiothepin or methysergide, were coadministered with serotonergic agonists to aggressive mice. If gepirone was acting through stimulation of postsynaptic receptor sites, then serotonin antagonists should decrease its effectiveness. If gepirone was acting by decreasing serotonergic impulse flow and thereby decreasing release of 5HT, then serotonin antagonists should exacerbate this effect and potentiate the actions of gepirone. The latter result was obtained and indicates that gepirone may be reducing aggression by decreasing serotonergic activity. It is known that reductions of serotonergic activity will mimic the actions of benzodiazepines in the conflict test (Tye et al. 1979; Iversen 1984). Whether decreased serotonergic activity induced by benzodiazepines is an important mechanism for their anxiolytic effects remains controversial (Iversen 1984; Thiebot et al. 1982, 1984). Reducing serotonergic activity increases predatory aggression (mouse killing by rats), but buspirone and gepirone neither inhibit muricidal behavior by established mouse killing rats nor induce muricidal behavior in non-muricidal rats (McMillen et al. submitted). Thus, data from intraspecies aggression may not be equated with results from interspecies (predatory) aggression.

It is obvious that gepirone and buspirone are complex drugs. Different effects can be obtained with different routes of administration or increasing dosage. These drugs potently displace [^{3}H]-5HT from hippocampal $5HT_{1a}$ binding sites and radiolabeled buspirone selectivity accumulates in the hippocampal formation. The saccharin-substituted buspirone analog, TVX Q 7821, shows similar behavior in its ability to bind to 5HT receptors (Dompert et al. 1985). Drugs without the long bulky alkyl chain do not show this subtle binding behavior: e.g. mCPP, TFMPP. Whether preferential stimulation of $5HT_{1a}$ receptors is important for the selective effects of the buspirone class of drugs on anxiety and aggression is as yet unclear. It is clear that as analogs of these drugs are studied it will be necessary to examine their effects on serotonergic impulse flow as well as 5HT binding sites. These results, also, suggest a strong linkage between serotonergic activity and anxiety and aggressive behaviors.

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