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Serotonergic Mechanisms in the Behavioral Effects of Buspirone and Gepirone

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EISON, A S , M S EISON, M STANLEY AND L A RIBLET *Serotonergic mechanisms in the behavioral effects of buspirone and gepirone* PHARMACOL BIOCHEM BEHAV **24**(3) 701–707, 1986 —The literature describing the role of serotonin (5-HT) in the mediation of anxiety is a controversial one Serotonergic involvement in the mechanism of action of two nonbenzodiazepine anxiolytics, buspirone and gepirone, supports a role for serotonin in anxiety The anticonflict effect of both drugs is blocked by serotonin lesions, and gepirone induces the serotonin syndrome A shift in the gepirone dose-response curve to the left in serotonin lesioned rats suggests that this may be 5-HT-receptor mediated Both buspirone and gepirone enhance the acoustic startle response and gepirone's effect is attenuated in serotonin lesioned animals While other components of buspirone's mechanism of action may suppress the behavioral expression of its serotonergic interactions, results from these studies suggest that serotonin agonist-like activity may be an important mechanism in the actions of a clinically proven nonbenzodiazepine anxiolytic (buspirone), and anxiolytic candidate (gepirone)

Serotonin Buspirone Acoustic startle 5,7-DHT lesion Nonbenzodiazepine Gepirone Serotonin syndrome Conflict behavior Diazepam Benzodiazepine

THE literature describing the role of serotonin (5-HT) in the generation and amelioration of anxiety has been a controversial one Apparently contradictory findings may result from a failure to clearly delimit whether one is studying anxiety, the pharmacology of imperfect animal models of anxiety, or the mechanism of action of anxiolytic drugs That alterations in serotonergic neurotransmission may modulate the perception of anxiety in man is suggested by recent clinical findings regarding the potential efficacy of serotonergic drugs in anxiety disorders Mianserin, which binds to 5-HT receptors [36] and antagonizes 5-HT-induced behaviors in animals [51] has been reported to possess anxiolytic properties in man [33] Similarly, the 5-HT-uptake inhibitor, clomipramine, has been used to treat panic attacks [25], while the more specific 5-HT-uptake inhibitor, zimelidine [35], appears promising in the treatment of phobic anxiety [17,30]

There is controversy regarding the role of 5-HT in commonly used animal models predictive of anxiolytic activity in man Models utilizing punishment-induced suppression of responding, or conflict tests, are quite sensitive to the actions of anxiolytic drugs [13] Behaviors suppressed by punishment may be released by depletion-induced [20,49], antagonist-induced [21,39], or lesion-induced [45,48] inactivation of 5-HT neurotransmission However, others have reported that 5-HT antagonists do not consistently affect punished responding [5,29], while 5-HT lesions may promote a profile of social interactions also observed following chronic benzodiazepine treatment [19]

While there is general agreement that the benzodiazepines do interact with brain 5-HT systems, the importance of these effects to their clinical actions has not been established Chlordiazepoxide injected into the dorsal raphe nucleus of rats attenuates the inhibition of responding induced by a signal associated with punishment [44] While the anticonflict effects of chlordiazepoxide are enhanced by inhibition of 5-HT synthesis [27], 5-HT lesions have been reported to block the anticonflict effects of benzodiazepine drugs [42,48] Diazepam [3] and chlordiazepoxide [41] enhance 5-HT release from brain slices which include the raphe nuclei, however, chlordiazepoxide reduces 5-HT release from striatal or nigral slices [41] and chronic diazepam treatment reduces cortical 5-HT turnover [4] Additionally, benzodiazepines reduce serotonergic unit activity in freely moving cats [47], and antagonize 5-HT agonist-induced hyponeophagia in rats [40]

A nonbenzodiazepine anxiolytic drug, buspirone, has been described which is clinically as efficacious as diazepam [18, 38, 53] yet lacks the pharmacological properties of the benzodiazepines which are ancillary to anxiolysis [43] Buspirone does not displace [^{3}H]benzodiazepines from benzodiazepine receptors *in vitro* [43] nor does it appear to work through direct benzodiazepine mechanisms [14] However, interactions with the serotonin system have been reported for buspirone [14,50] and for an analogue lacking buspirone's *in vitro* affinity for dopamine receptors, gepirone [12] (see Fig 1) As many investigations of the role of serotonin in anxiety have focused upon interactions between ben-

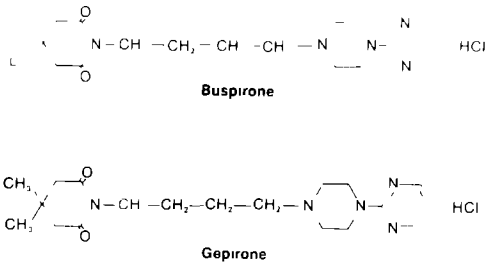

FIG 1 Chemical structures of buspirone and gepirone

zodiazepines and serotonin systems, it was of interest to determine whether alterations in serotonergic neurotransmission contribute to the behavioral pharmacology of non-benzodiazepine anxiolytic drugs and drug candidates

METHOD

Male albino Sprague-Dawley rats (250–350 g, Charles River, MI) were housed in groups of eight in standard laboratory cages With the exception of animals used in conflict experiments (see Behavioral Tests) rats were maintained in a temperature and humidity-controlled room on a 12/12 light/dark cycle (lights on 6 00 a m) with free access to food and water

Surgical Procedures

Serotonin lesions were produced in rats by administration of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) via indwelling intraventricular polyethylene cannulae Methods were adapted from Noble *et al* [34] Rats were anesthetized with methoxyflurane and placed in a stereotaxic instrument (D Kopf, Tujunga, CA) Cannulae made from polyethylene tubing (PE 10, Intramedic, 50 mm length) were inserted through burr holes (Anterior-Posterior 1 0 mm behind bregma, Medial-Lateral ±1 5 mm from midsaggital suture) into the lateral ventricles The length of the intracerebral portion of each cannula was 3 5–4 0 mm ventral from skull surface Bone screws were secured on the skull and both the cannulae and screws were fixed to the skull with dental acrylic cement

Lesions were made following a five day post-surgery recovery period Rats were pretreated with desipramine (25 mg/kg) to prevent uptake of the neurotoxin into noradrenergic neurons [22] Forty-five min later pentobarbital (15 mg/kg) was administered to prevent convulsions Fifteen min later rats received 150 μg of 5,7-dihydroxytryptamine (free base) dissolved in 20 μl of vehicle (0 9% NaCl and 0 1% L-ascorbic acid) The 5,7-DHT solution was slowly infused via the polyethylene cannulae (10 μl per side) using a Hamilton microsyringe

Lesioned rats were studied during the supersensitive post-lesion phase to rigorously investigate potential serotonin agonist properties of the drugs Consistent with the commonly accepted time course for neuronal degeneration and the development of serotonergic supersenstivity [46], 5,7-DHT-treated rats were tested in the serotonin syndrome, conflict and acoustic startle tests between 7 and 16 days after lesioning As the behavior of naive control rats did not differ significantly from that of vehicle-injected controls, these data were pooled for analysis

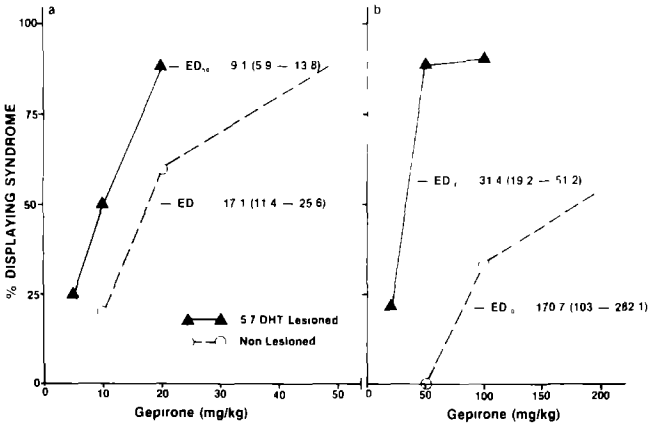

FIG 2 Ability of various doses of gepirone administered intraperitoneally (a) or orally (b) to produce the serotonin syndrome in control or 5,7-DHT-lesioned rats The number of animals tested at each dose ranged from 8 to 10, each point represents the percentage of rats displaying the syndrome at each dose of gepirone ED_{50} values are presented with 95% fiducial limits in parenthesis

Behavioral Tests

Serotonin syndrome Rats were placed in square plastic cages (dimensions L×W×H were 37×27×10 cm, respectively) with metal screen lids and a layer of fresh sawdust covering the floor The serotonin syndrome was quantified by recording the presence or absence of the following signs resting tremor (especially of the head and forepaws), muscular hypertonus (evaluated by flexing the rat's hindlimbs), hindlimb abduction (a posturing of the hindlimbs forward toward the forelimbs), Straub tail (rigid or corkscrew tail), lateral head weaving (slow side to side movements of the head) and reciprocal forepaw treading (continuous dorsoventral movements of the forepaws) If at least 4 of these 6 signs were observed, the syndrome was rated as "present " Applying these strict criteria, the syndrome may be regarded as a behavioral model for the study of functional activation of central 5-HT systems [28] Preliminary studies revealed that gepirone exhibited reliable activity in this test Therefore, equal doses of buspirone and diazepam were also studied In naive rats these doses were 50, 100, 200 mg/kg, PO and 10, 20, 50 mg/kg, IP In lesioned rats the following doses were tested 20, 50, 100 mg/kg, PO and 5, 10, 20 mg/kg, IP Following habituation to the test cages, either buspirone, gepirone or diazepam were administered and rats were rated for the presence or absence of the 5-HT syndrome every 5 min for 50 min

A dose response relationship for the L-5-hydroxytryptophan-induced serotonin syndrome was obtained in control rats Behavioral supersensitivity in lesioned rats was confirmed by quantifying their response to doses of L-5-HTP which were subthreshold in control animals Of the anxiolytics tested, only gepirone produced the 5-HT behavioral syndrome in control rats, consequently, the ability of gepirone to induce the serotonin syndrome was evaluated in 5,7-DHT-treated rats

Conflict test Rats were tested in a standard rodent operant test chamber (30×25×34 cm, Coulborn Instruments, Allentown, PA) with a grid floor through which a scrambled

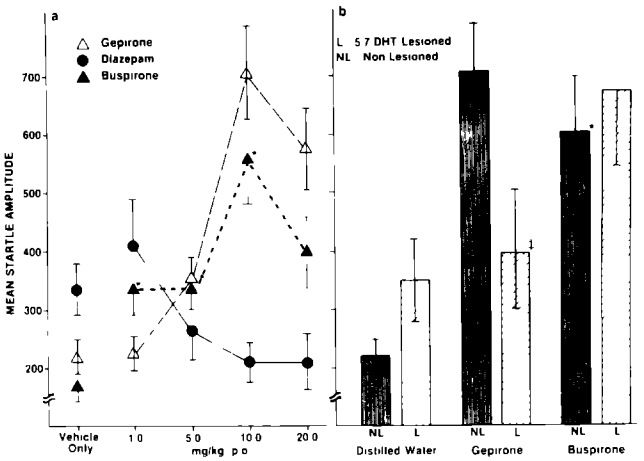

FIG 3 (a) Effects of various oral doses of buspirone, gepirone and diazepam on the amplitude of the acoustic startle reflex Each value represents the mean (±SEM) obtained from 8 animals (b) Effects of the peak dose of gepirone and buspirone (10 mg/kg, PO) on the amplitude of the startle reflex in control and 5,7-DHT-lesioned rats (N=8 per group) Statistically significant from corresponding vehicle-treated groups, $*p<0.05$ and from non-lesioned groups, $\ddagger p<0.05$ (Student's t-test)

TABLE 1

CONCENTRATION OF SEROTONIN (5-HT) AND 5-HYDROXYINDOLEACETIC ACID (5-HIAA) IN CONTROL AND 5,7-DHT-LESIONED RATS IN SELECTED BRAIN REGIONS

Region	Treatment	Concentration† (μg/mg tissue) 5-HT	5-HIAA
Control	Frontal Cortex	326 ± 23	309 ± 18
5,7-DHT Lesion	Frontal Cortex	36 ± 5*	31 ± 5*
(% Control)		(11 0)	(10 0)
Control	Striatum	359 ± 16	558 ± 29
5,7-DHT Lesion	Striatum	83 ± 13*	88 ± 14*
(% Control)		(23 1)	(15 8)

*$p<0.001$, significantly different from control, Student's t-test
†Concentrations reflect mean ± S E M for each set of determinations

electric shock (0 2 mA for 1 sec) could be delivered Cages were equipped with water bottles and licks at the water spout were detected by interruption of a photocell beam The test chamber was enclosed in a darkened, sound-attenuating, ventilated box, which prevented distraction of the animals A modification of the Vogel conflict test was used [52] As previous studies revealed that rats are most sensitive to the anticonflict effects of these drugs during their night cycle [14], rats housed in a reversed day-night room (lights off 4 a m , on 4 p m) were bottle trained by water depriving them for 24 hr and then permitting 15 min free access to the water spout in the test chamber Animals were then water deprived for an additional 23½ hr prior to testing

Both control and 5,7-DHT-treated rats were orally administered either buspirone (10 mg/kg), gepirone (10 mg/kg), diazepam (10 mg/kg) or vehicle (distilled water or methocel) 15 min prior to testing Rats were subsequently placed in the test chamber, any rat that did not exhibit at least 20 licks during the first 5 min of the test session was eliminated from the data pool, otherwise the 20th lick triggered a 5 min test session Total licks at the water spout in the presence of an electric shock contingency (every 20th lick, 1 shock) were recorded

Acoustic startle test Startle monitoring cages (30×17×19 cm, Columbus Instruments, Columbus, OH) were used to record the amplitude of the rats' startle reflex Cages were housed in a dark, ventilated, sound-attenuated chamber, each was placed 20 cm from a high frequency speaker The floor of the cage consisted of a steel grid suspended on a force transducer The startle response, reflected in the startle amplitude, was defined as the maximum displacement of the force transducer occurring during the first 200 msec following the presentation of an acoustic stimulus Amplitude of the startle reflex is expressed in units which reflect a voltage proportional to the force the subject exerted against the cage floor The startle stimulus was a 4000 Hz tone of 500 msec duration and 106 dB amplitude White noise was maintained at 48 dB After oral dosing, subjects were placed in a holding cage for 5 min, immediately following this, subjects were habituated to the test cages for 5 min after which the test began The test consisted of a series of ten stimuli presented with a 90 sec inter-stimulus-interval from which the mean startle amplitude was derived

Dose response curves for buspirone, gepirone and diazepam were obtained in non-lesioned rats As only gepirone and buspirone produced alterations in the basal startle reflex, these drugs were investigated at their maximally effective dose in 5,7-DHT-treated rats

Drugs

Buspirone HCl and gepirone HCl (Bristol-Myers, Evansville, IN) were dissolved in distilled water Diazepam (Hoffman-LaRoche, Nutley, NJ) was prepared as a suspension L-5-hydroxytryptophan (Sigma Chemical Co , St Louis, MO) was dissolved in distilled water with warming and the addition of dilute hydrochloric acid 5,7-Dihydroxytryptamine creatinine sulfate was obtained from Sigma Chemical Co Desipramine HCl (Lakeside Laboratories, Milwaukee, WI) and sodium pentobarbital (Abbott Laboratories, North Chicago, IL) were dissolved in distilled water All compounds were administered in a volume of 10 ml/kg, orally and 1 ml/kg, intraperitoneally Methoxyflurane (Pitman-Moore, Washington Crossing, NJ) was administered by inhalation (0 2 ml total volume over 30 min) from cotton gauze placed beneath the animal's snout

Monoamine Analysis

Twenty-four hours following the completion of behavioral testing, 5,7-DHT-treated and control rats were decapitated, their brains quickly removed, frontal cortices and striata dissected on ice and stored at −80°C until assay Cortical concentrations of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), and the dopamine metabolite homovanillic acid (HVA) were analyzed by high pressure liquid chromatography with electrochemical detection [32]

Tissue samples were weighed, then homogenized (Polytron, Brinkmann Instruments) in 500 μl of 0 1 M $HClO_4$ which contained 60 ng/ml N-methyl-serotonin as the internal stand-

ard Homogenates were centrifuged at 25,000×g for 10 min, the supernatant decanted into new plastic tubes and recentrifuged The clear supernatant was used for analysis

Chromatographic analysis was conducted using a Waters 6000A pump, a reverse phase (C-18) Altex Ultrasphere I P column, a LC-4A amperometric detector with a TL-5 glassy carbon electrode and an Ag/AgCl reference electrode (Bioanalytical Systems) The amperometric detector oxidizing potential was maintained at 0 70 V The mobile phase, pumped at a flow rate of 1 0 ml/min, consisted of a mixture of 0 1 M sodium acetate buffer (pH 4 6) containing 7% methanol and 0 1 mM EDTA

Statistics

ED_{50} values were calculated by the method of Berkson [2] Data from conflict and acoustic startle tests were analyzed by means of a Student's *t*-test

RESULTS

Serotonin Syndrome

While buspirone and diazepam failed to induce the serotonin syndrome in non-lesioned rats, gepirone and L-5-HTP were active in this test Gepirone elicited the serotonin syndrome in non-lesioned rats within 10 min by either intraperitoneal or oral routes After intraperitoneal administration, gepirone was approximately 12-fold more potent than L-5-HTP in producing this reponse However, while L-5-HTP animals exhibited all six of the syndrome behaviors, gepirone-treated rats only displayed tremor, Straub tail, robust muscular hypertonus, minimal treading and hindlimb abduction At high doses of gepirone, the animals' hindlimbs appeared rigidly locked in a splayed position As presented in Fig 2, 5,7-DHT-treated rats displayed a marked supersensitivity to both the oral and intraperitoneal injection of gepirone When compared to nonlesioned controls, gepirone was 1 8-fold more potent in inducing the syndrome in 5,7-DHT-treated rats following intraperitoneal administration and 5 4-fold more potent after oral administration 5,7-DHT-treated rats exhibited the syndrome at very low doses of L-5-HTP The ED_{50} (in mg/kg with 95% confidence limits) for L-5-HTP induction of the syndrome was 217 5 (167 4–282 7) in control rats and 21 2 (13 3–33 6) in 5,7-DHT-treated animals Latency to onset of the serotonin syndrome decreased in 5,7-DHT-treated rats While control rats displayed the syndrome 20–30 min after L-5-HTP and 10–15 min after gepirone, it occurred within 5 min in 5,7-DHT-treated rats after either drug

Conflict Test

Buspirone, gepirone and diazepam (all 10 mg/kg) were all active in the conflict procedure when compared to vehicle-treated controls ($p<0$ 05) Buspirone administration resulted in a 5-fold increase in licking despite the electroshock contingency, while equal doses of gepirone and diazepam induced 3 8 and 1 9-fold increases, respectively While the performance of 5,7-DHT-treated rats administered vehicle did not differ significantly from that of vehicle-treated controls (mean total licks±s e m = 47 1±3 4 and 49 8±6 6, respectively), 5,7-DHT-treated rats licked at the spout significantly less than non-lesioned rats administered identical doses of buspirone, gepirone or diazepam (mean licks±s e m = 59 8±8 9, 45 8±4 7, 70 1±17 1, respectively) Significant anticonflict activity was not induced by any of the three compounds tested in 5,7-DHT-treated rats

Acoustic Startle Test

Buspirone and gepirone increased the amplitude of the acoustic startle reflex relative to vehicle-only controls ($p<0$ 05), while diazepam did not (see Fig 3a) Buspirone and gepirone-induced increases in the startle response paralleled each other in a dose-responsive manner, with the peak effect occurring at 10 mg/kg, PO The amplitude of gepirone's effects exceeded that of buspirone only at the 20 mg/kg dose When the peak dose was tested in rats with serotonin lesions, gepirone's effects upon the startle reflex were significantly attenuated ($p<0$ 05) while buspirone's effects remained unchanged (see Fig 3b)

Monoamine Analysis

Table 1 shows the extent of regional depletions of central 5-HT and 5-HIAA Neurochemical determinations revealed 5,7-DHT-treated rats to have at least 90% depletions of 5-HT and 5-HIAA in the frontal cortex Striatal depletions of 5-HT and its metabolite in these rats were approximately 80% Regional levels of the dopamine metabolite HVA only marginally differed in lesion and control animals (82% of control), suggesting specificity of the lesion

DISCUSSION

The results of this study suggest that serotonergic mechanisms play a role in the behavioral effects of the nonbenzodiazepine anxiolytic buspirone, and the anxiolytic candidate, gepirone

Previous studies have established that the complex behavioral syndrome observed following administration of serotonin agonists reflects activation of central serotonin receptors [28], particularly the 5-HT_1 receptor [31] The present study demonstrates that gepirone induces the serotonin syndrome following either oral or intraperitoneal administration Gepirone has been reported to bind *in vitro* to rat hippocampal 5-HT_1 receptors (D P Taylor, personal communication), and down-regulates 5-HT_2 receptors following chronic administration [15] That gepirone's induction of the serotonin syndrome is 5-HT receptor mediated is suggested by the observation that the gepirone dose-response curve is shifted to the left in behaviorally supersensitive serotonin-lesioned rats

Trulson *et al* [46] demonstrated enhanced behavioral sensitivity to the serotonin precursor and serotonin agonist-induced syndrome in 5,7-DHT treated rats Lesion-induced changes in responsivity were greater in response to L-5-HTP than to direct acting agonists such as 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) Similarly, in the present study, L-5-HTP exhibited a greater dose-response shift than gepirone in serotonin-lesioned rats Reduction in the latency to onset of the serotonin syndrome is also characteristic of supersensitive lesioned animals [46] and was observed in L-5-HTP and gepirone-treated lesioned rats in the present study Interestingly, the magnitude of the shift of the dose-response curve to gepirone in lesioned animals is identical to that reported by Trulson *et al* [46] for 5-MeODMT While this observation does not directly address the synaptic mechanisms by which gepirone exerts its serotonergic effects, it is important to note that gepirone

does not possess serotonin re-uptake blocking properties (D P Taylor, personal communication)

These data suggest that gepirone may be acting as a direct serotonin agonist This is supported by the observation that gepirone induces contralateral rotation in rats with unilateral serotonin lesions [12] and that the gepirone-induced serotonin syndrome is blocked by pretreatment with the serotonin antagonist methysergide (A S Eison, unpublished observation) In contrast to gepirone, neither buspirone nor diazepam induced the serotonin syndrome Our results with buspirone contrast with a recent anecdotal report that buspirone does induce the serotonin syndrome [26] Differences in rat strain and handling of the animals may contribute to these disparate results However, in the present study, buspirone failed to elicit the syndrome even at doses as high as 200 mg/kg, IP, in demonstrably supersensitive lesioned animals The failure of buspirone to induce the serotonin syndrome in the present study is particularly interesting because buspirone has been reported to bind to 5-HT_1 receptors in rat and calf hippocampus [24,37]

Buspirone, gepirone, and diazepam were all active in conflict tests, and their activity was attenuated by serotonin lesions These diazepam results are consistent with reports that serotonin lesions block the anticonflict effects of benzodiazepines [42,48] We now report that the anticonflict action of the nonbenzodiazepines buspirone and gepirone are similarly blocked by disruptions of serotonergic neurotransmission

However, other studies have reported that anticonflict action may be induced by manipulations which reduce 5-HT neurotransmission [20, 39, 45, 48, 49] The benzodiazepines [47], buspirone [50] and gepirone (C P VanderMaelen, personal communication) reduce the activity of serotonergic neurons in the dorsal raphe nucleus It has also been reported that serotonin precursors and agonists may have a proconflict effect, reducing punished responding in conflict tests [39,45] Recently, Engel *et al* [16] demonstrated that subchronic treatment with the 5-HT synthesis inhibitor p-chlorophenylalanine (PCPA) produces anticonflict effects in the Vogel paradigm, and that this effect is antagonized by the putative 5-HT agonist 8-hydroxy-2(di-n-propylamino)-tetralin (8-OH-DPAT) PCPA pretreatment also increased sensitivity to the suppressant effects of L-5-HTP upon punished behavior

Engel *et al* [16] suggest that the attenuated anticonflict effect of 8-OH-DPAT in presumably supersensitive PCPA-treated rats reflects 5-HT agonist activity The attenuated anticonflict effects of buspirone and gepirone in rats made supersensitive by neurotoxin lesions in the present study may therefore reflect serotonin agonist activity

As has been reported by others [7], diazepam did not affect the basal acoustic startle response in the present study However, as presented in Fig 3, both gepirone and buspirone produced dose-dependent increases in the the amplitude of this response Several studies have suggested that the serotonin system plays an important role in modulating the acoustic startle reflex Reductions in serotonergic neurotransmission produced by lesions of midbrain serotonin neurons [10] or by inhibition of serotonin synthesis [6] have been observed to increase the amplitude of the startle response, while enhanced serotonergic neurotransmission reduces the startle response [11,23]

Low doses of the hallucinogenic drugs lysergic acid diethylamide (LSD) and N,N-dimethyltryptamine (DMT) inhibit serotonin-containing raphe neurons [1] and enhance the startle response [8,9] Similarly, both buspirone and gepirone increase the startle response and produce marked inhibitions of activity in dorsal raphe neurons (0 11 and 0 25 mg/kg, IV, respectively, C P VanderMaelen, personal communication) Further, buspirone enhances the startle reflex within the dose range observed to inhibit serotonin cell firing when intragastrically administered (1–20 mg/kg) (see Fig 3)

In contrast, high doses of LSD and DMT which presumably inhibit neurons postsynaptic to dorsal raphe cells suppress the startle response [8,9] Interestingly, at oral doses of 20 mg/kg or greater both buspirone and gepirone's enhancement of acoustic startle is diminished, and begins to return to control levels

While in the present study a significant enhancement of acoustic startle in serotonin lesioned rats was not observed, the startle potentiating effects of gepirone were attenuated by serotonin lesions As these animals were tested at a time of demonstrated supersensitivity, it is possible that the gepirone-induced reduction in the startle response reflects postsynaptic serotonergic agonist activity Interestingly, the acoustic startle response of buspirone-treated rats was not altered by serotonin lesions This may suggest that buspirone is a weaker postsynaptic 5-HT agonist than gepirone, or that some other component of buspirone's mechanism of action modifies the behavioral expression of its serotonergic actions This may also account for buspirone's inability to induce the serotonin syndrome in naive and 5,7-DHT-treated animals Other aspects of buspirone's pharmacology [14], perhaps its dopaminergic interactions, may attenuate the expression of its serotonergic properties despite electrophysiological and biochemical evidence of 5-HT-agonist-like activity

The present study has therefore demonstrated differential roles of serotonin in the behavioral effects of the nonbenzodiazepines buspirone and gepirone, and suggests that the integrity of brain serotonin systems influences the anticonflict effects of these nonbenzodiazepine drugs

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