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Behavioral Studies with Anxiolytic Drugs. III. Antipunishment Actions of Buspirone in the Pigeon Do Not Involve Benzodiazepine Receptor Mechanisms[1]

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ABSTRACT

Buspirone, a clinically effective anxiolytic, has not shown robust effects consistently in procedures used traditionally with rodents and nonhuman primates to evaluate potential antianxiety activity. When key pecking by pigeons was maintained by food and was punished alternately under one component of a multiple schedule by the presentation of electric shock (conflict procedure), buspirone (0.03–10.0 mg/kg i.m.) produced increases in punished responding that were up to 30 times those of the control response rate. These doses did not affect or decreased unpunished responding. A buspirone analog, MJ 13805 (gepirone) produced effects similar to buspirone, although unpunished responding was slightly more sensitive to the rate-decreasing effects of MJ 13805 than to those of buspirone. A metabolite of buspirone, 1-pyrimidinyl piperazine (1-PP; MJ 13653), did not affect key pecking across a wide dose range (0.01–3.0 mg/kg i.m.), although slight decreases in both punished and unpunished responding occurred at the highest dose. Increases in punished responding with buspirone were not affected by the benzodiazepine receptor antagonist Ro 15-1788 (0.01–0.1 mg/kg i.m.). [^{3}H] Diazepam binding to pigeon cerebrum or cerebellum *in vivo* was not altered by buspirone, or did buspirone, MJ 13805, or 1-pyrimidinyl piperazine displace [^{3}H]flunitrazepam binding *in vitro* at pharmacologically relevant concentrations. These findings confirm previous work demonstrating marked rate-increasing effects of buspirone on punished responding in the pigeon, extend such effects to the buspirone analog MJ 13805 and indicate that the effects of buspirone are not mediated through the benzodiazepine receptor complex.

Buspirone (8[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro[4.5] decane-7, 9-dione), is a novel nonbenzodiazepine antianxiety agent with a number of unique properties. In contrast to most anxiolytics, buspirone lacks anticonvulsant, sedative-hypnotic and muscle relaxant properties (Riblet *et al.*, 1983; Rickels *et al.*, 1982; Stanton *et al.*, 1981). Furthermore, buspirone appears to be inactive in benzodiazepine or GABA binding assays (Riblet *et al.*, 1982; Stanton *et al.*, 1981), although evidence for some activity (*in vivo*) at the benzodiazepine-GABA ionophore complex has been demonstrated (Oakley and Jones, 1984; Garattini *et al.*, 1982; Weissman *et al.*, 1984a). The distinct pharmacological profile of buspirone is further emphasized by the findings that buspirone may affect both dopaminergic and serotonergic systems (Glaser and Traber, 1983; Hjorth and Carlsson, 1982; McMillen and Mattiace, 1983; Skolnick *et al.*, 1985; Taylor *et al.*, 1982).

Although clinically effective as an antianxiety compound (Goldberg and Finnerty, 1979, 1982; Rickels *et al.*, 1982), buspirone is far less efficacious than other anxiolytics (*e.g.*, the benzodiazepines chlordiazepoxide and diazepam) in traditional punishment procedures using rats or squirrel monkeys that are predictive of anxiolytic action in man (J. E. Barrett and J. M. Witkin, in preparation; Lippa *et al.*, 1977; Sepinwall and Cook, 1978; Weissman *et al.*, 1984a). Recently, it has been demonstrated that, in pigeons, buspirone produces marked increases in punished responding that are comparable to those obtained after benzodiazepines (Witkin and Barrett, 1986). These increases were not altered by coadministration of the dopaminergic compounds apomorphine or haloperidol, suggesting that the dopamine system does not contribute directly to the antianxiety or anticonflict actions of buspirone in this species.

The present experiments further examined the effects of buspirone on punished behavior of pigeons with specific emphasis on the potential involvement of benzodiazepine receptor mechanisms mediating these effects. Both behavioral studies, focusing on the possible antagonism of the effects of buspirone by the specific benzodiazepine receptor antagonist Ro 15-1788, and *in vivo* and *in vitro* neuropharmacological studies were

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ABBREVIATIONS: GABA, γ-aminobutyric acid; 1-PP, 1-pyrimidinyl piperazine.

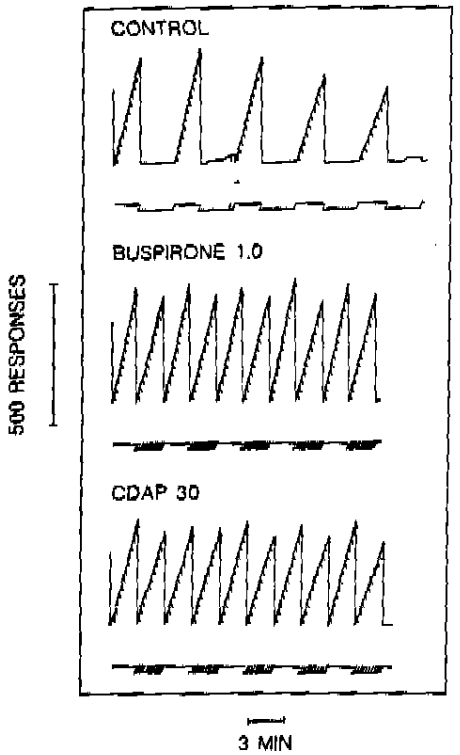

Fig. 1. Cumulative response records of control performance and effects of buspirone (1.0 mg/kg) and chlordiazepoxide (CDAP, 30.0 mg/kg) under the multiple schedule. Ordinates: cumulative responses; abscissae: time. Components in which responding was punished are denoted by a deflection of the event pen beneath each record. Food delivery is indicated by the displacement of the response pen; shock presentations are indicated by the momentary deflection of the event pen beneath each record. The pen was reset to baseline at the end of each 3-min component.

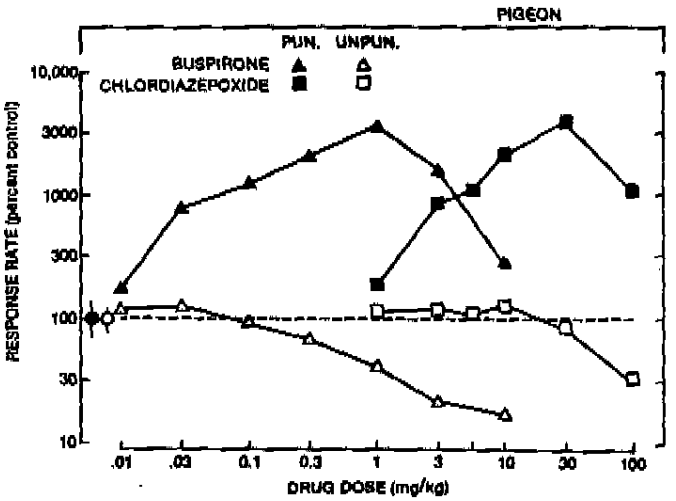

Fig. 2. Dose-response curves for chlordiazepoxide and buspirone on unpunished and punished responding. Unconnected symbols on the left represent control measures ±1 S.E. Figures are based on data from 5 pigeons with 7 to 12 control measures from each subject. Horizontal dashed line indicates no effect. Drug effects on punished responding are indicated by filled symbols; effects on unpunished responding by open symbols.

chlordiazepoxide (100.0 mg/kg) decreased responding significantly for all pigeons.

Figure 1 shows cumulative response records that are typical of effects of buspirone and chlordiazepoxide. Both drugs produced marked, sustained increases in the rate of punished responding with little alteration of unpunished responding. Rates and patterns of responding under the two components were very similar after drug administration. These effects were apparent at the beginning of each session and persisted throughout the session.

The effects of buspirone on punished and unpunished responding were not altered by the benzodiazepine antagonist Ro 15-1788 (fig. 3). When administered alone, doses of 0.01 and 0.03 mg/kg of Ro 15-1788 produced marginal effects; the highest dose (0.1 mg/kg) produced slight decreases in both unpunished and punished responding. The combined administration of Ro 15-1788 and buspirone produced effects on unpunished behavior that were similar to those obtained with buspirone alone. When combined with intermediate doses of buspirone (0.1–1.0

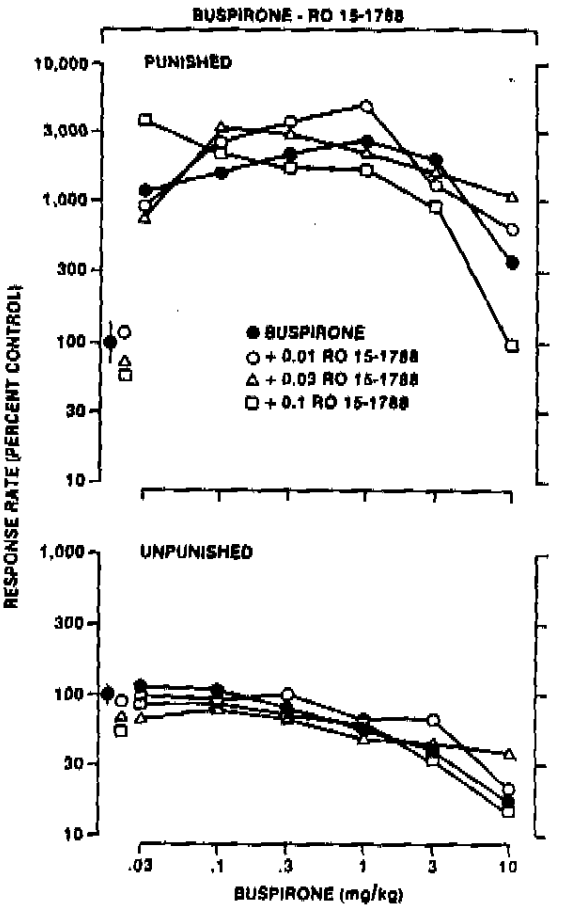

Fig. 3. Dose-response curves for buspirone, Ro 15-1788 and their interaction on punished and unpunished responding. Unconnected filled symbols on left denote control performance expressed as a percentage of control values; unconnected open symbols represent effects of Ro 15-1788 alone. Data are based on 5 pigeons with at least 9 control measures from each subject. Connected filled symbols are effects of buspirone given alone or with the coadministration of saline; connected symbols represent interactions of Ro 15-1788 with buspirone. Data from punished responding are shown in the upper panel; data from unpunished responding in the lower panel.

anxiolytic actions of buspirone (McMillen *et al.*, 1983; Sanghera and German, 1983; Temple *et al.*, 1982). However, in behavioral studies conducted to date, it has not been possible to demonstrate either a potentiation or antagonism of buspirone's effects on punished responding with dopamine agonists or antagonists (Witkin and Barrett, 1986). Both the buspirone analog MJ 13805 and the buspirone metabolite 1-PP have been reported to be devoid of dopaminergic activity (Garattini *et al.*, 1982; McMillen and Mattiace, 1983).

In the present experiments, MJ 13805 and buspirone produced similar effects on punished responding in pigeons, whereas 1-PP exerted little activity suggesting that, at least in the pigeon, 1-PP does not appear to be an active metabolite. In support of this finding, studies of plasma levels of 1-PP in pigeons after buspirone or MJ 13805 administration have shown that this species does not convert these compounds to 1-PP (D. Taylor, personal communication). The lack of activity of 1-PP in the pigeon would appear to argue against the explanation that buspirone's effects may be due to the activity of 1-PP without direct effects on the dopaminergic system (Garattini *et al.*, 1982).

At present, the mechanisms by which buspirone exerts its anticonflict and anxiolytic activities remain elusive. Recently, it has been suggested that serotonergic systems may be involved in mediating the activity of buspirone (Eison *et al.*, 1986; Glaser and Traber, 1983; Hjorth and Carlsson, 1982). However, both behavioral effects of buspirone remains ambiguous (J. M. Witkin, J. E. Barrett, R. S. Mansbach, G. T. Bolger, B. A. Weissman and P. Skolnick, submitted manuscript). Nevertheless, parenterally administered buspirone has been shown to block apomorphine-induced stereotypy, to inhibit the 5-hydroxytryptamine syndrome induced by 5-methoxy-N,N-dimethyltryptamine and to delay *p*-chloroamphetamine-elicited behavior (Skolnick *et al.*, 1984). Further studies of a combined neuropharmacological and behavioral nature are required to elucidate the mechanisms of this unique compound.

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References

AZRIN, N.: A technique for delivering shock to pigeons. J. Exp. Anal. Behav. 2: 161–163, 1959.

BOLGER, G. T., WEISSMAN, B. A., LUEDDENS, H., BASILE, A. S., MANTIONE, C. R., BARRETT, J. E., WITKIN, J. M., PAUL S. M. AND SKOLNICK, P.: Late evolutionary appearance of "peripheral-type" binding sites for benzodiazepines. Brain Res. 338: 366–370, 1985.

EISON, A. S., EISON, M. S., STANLEY, M. AND RIBLET, L. A.: Serotonergic mechanisms in the behavioral effects of buspirone and gepirone. Pharmacol. Biochem. Behav. 24: 701–707, 1986.

FERSTER, C. B.: The use of the free operant in the analysis of behavior. Psychol. Bull. 50: 263–274, 1953.

FERSTER, C. B. AND SKINNER, B. F.: Schedules of Reinforcement, Appleton-Century-Crofts, New York, 1957.

GARATTINI, S., CACCIA, S. AND MENNINI, T.: Notes on buspirone mechanism of action. J. Clin. Psychiatry 43: 19–22, 1982.

GELLER, I. AND HARTMANN, R. J.: Effects of buspirone on operant behavior of laboratory rats and cynomologus monkeys. J. Clin. Psychiatry 43: 25–32, 1982.

GLASER, T. AND TRABER, J.: Busipirone: Action on serotonin receptors in calf hippocampus. Eur. J. Pharmacol. 88: 137–138, 1983.

GOLDBERG, H. L. AND FINNERTY, R. J.: The comparative efficacy of buspirone and diazepam in the treatment of anxiety. Am. J. Psychiatry 136: 1184–1187, 1979.

GOLDBERG, H. L. AND FINNERTY, R. J.: Comparison of buspirone in two separate studies. J. Clin. Psychiatry 43: 87–91, 1982.

HJORTH, S. AND CARLSSON, A.: Buspirone: Effects on central monoaminergic transmission—Possible relevance to animal experimental and clinical findings. Eur. J. Pharmacol. 83: 299–303, 1982.

LIPPA, A. S., GREENBLATT, E. N. AND PELHAM, R. W.: The use of animal models for delineating the mechanisms of action of anxiolytic agents. *In* Animal Models in Psychiatry and Neurology, ed. by I. Hanin and E. Usdin, pp. 279–292, Pergamon Press, New York, 1977.

MCMILLEN, B. A. AND MATTIACE, L. A.: Comparative neuropharmacology of buspirone and MJ 13805, a potential anti-anxiety drug. J. Neural. Transm. 57: 255–265, 1983.

MCMILLEN, B. A., MATTHEWS, R. T., SANGHERA, M. K., SHEPARD, P. D. AND GERMAN, D. C.: Dopamine receptor antagonism by the novel anti-anxiety drug, buspirone. J. Neurosci. 3: 733–738, 1983.

OAKLEY, N. R. AND JONES, B. J.: Buspirone enhances [^{3}H]flunitrazepam binding *in vivo*. Eur. J. Pharmacol. 87: 499–500, 1984.

RIBLET, L. A., EISON, A. S., EISON, M. S., NEWTON, R. E., TAYLOR, D. P. AND TEMPLE, D. L.: Buspirone: An anxioselective alternative for the management of anxiety disorders. Prog. Neuropsychopharmacol. Biol. Psychiatry 7: 663–668, 1983.

RIBLET, L. A., TAYLOR, D. P., EISON, M. S. AND STANTON, H. C.: Pharmacology and neurochemistry of buspirone. J. Clin. Psychiatry 43: 11–16, 1982.

RICKELS, K., WEISSMAN, K., NORSTAD, N., SINGER, M., STOLTZ, D., BROWN, A. AND DANTON, J.: Buspirone and diazepam in anxiety. J. Clin. Psychiatry 43: 81–86, 1982.

SANGHERA, M. K. AND GERMAN, D. C.: The effects of benzodiazepine and nonbenzodiazepine anxiolytics on locus coeruleus unit activity. J. Neural. Transm. 57: 267–279, 1983.

SEPINWALL, J. AND COOK, L.: Behavioral pharmacology of antianxiety drugs. *In* Handbook of Psychopharmacology, ed. by L. L. Iversen, S. D. Iversen and S. H. Snyder, vol. 13, pp. 345–393, Plenum Press, New York, 1978.

SKOLNICK, P., PAUL, S. M. AND WEISSMAN, B. A.: Preclinical actions of buspirone. Pharmacotherapy 4: 308–314, 1984.

SKOLNICK, P., WEISSMAN, B. A. AND YOUDIM, M. B. H.: Monoaminergic involvement in the pharmacologic action of buspirone. Br. J. Pharmacol. 86: 637–644, 1985.

STANTON, H. C., TAYLOR, D. P. AND RIBLET, L. A.: Buspirone—An anxioselective drug with dopaminergic action. *In* Neurobiology of the Nucleus Accumbens, ed. by R. B. Chronister and J. F. DeFrance, pp. 316–319, Haer Institute, Brunswick, Maine, 1981.

TAYLOR, D. P., ALLEN, L. E., BECKER, J. A., CRANE, M., HYSLOP, D. AND RIBLET, L. A.: Changing concepts of the biochemical action of the anxioselective drug, buspirone. Drug Dev. Res. 4: 95–108, 1984.

TAYLOR, D. P., RIBLET, L. A., STANTON, H. C., EISON, A. S., EISON, M. S. AND TEMPLE, D. L., JR.: Dopamine and antianxiety activity. Pharmacol. Biochem. Behav. 17: suppl. 1, 25–35, 1982.

TEMPLE, D. L., YEVICH, J. P. AND NEW, J. S.: Buspirone: Chemical profile of a new class of anxioselective agents. J. Clin. Psychiatry 43: 4–9, 1982.

WEISSMAN, B. A., BARRETT, J. E., BRADY, L. S., WITKIN, J. M., MENDELSON, W. B., PAUL, S. M. AND SKOLNICK, P.: Behavioral and neurochemical studies on the anticonflict actions of buspirone. Drug Dev. Res. 4: 83–93, 1984a.

WEISSMAN, B. A., BOLGER, G. T., ISSAC, L., PAUL, S. M. AND SKOLNICK, P.: Characterization of the binding of [^{3}H] Ro5-4864, a convulsant benzodiazepine, to guinea pig brain. J. Neurochem. 42: 969–975, 1984b.

WILLIAMSON, M., PAUL, S. AND SKOLNICK, P.: Labelling of benzodiazepine receptors *in vivo*. Nature (Lond.) 275: 551–553, 1978.

WITKIN, J. M. AND BARRETT, J. E.: Behavioral effects and benzodiazepine antagonist activity of Ro 15-1788 (flumazepil) in pigeons. Life Sci. 37: 1587–1595, 1985.

WITKIN, J. M. AND BARRETT, J. E.: Interaction of buspirone and dopaminergic agents on punished behavior of pigeons. Pharmacol. Biochem. Behav. 24: 751–756, 1986.

WITKIN, J. M., BARRETT, J. E., COOK, J. M. AND LARSCHEID, P.: Differential antagonism of diazepam-induced loss of the righting response. Pharmacol. Biochem. Behav. 24: 963–965, 1986.

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