Pharmacology Biochemistry & Behavior, Vol. 32, pp. 227–232. Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Neurochemical Changes in Pigeon Cerebrospinal Fluid During Chronic Administration of Buspirone or 8-Hydroxy-2-(di-*n*-Propylamino)Tetralin (8-OH-DPAT)

MICHAEL A. NADER[1] AND JAMES E. BARRETT

Behavioral Pharmacology Laboratory, Department of Psychiatry
Uniformed Services University of the Health Sciences
4301 Jones Bridge Road, Bethesda, MD 20814-4799

Received 8 April 1988

NADER, M. A. AND J. E. BARRETT. *Neurochemical changes in pigeon cerebrospinal fluid during chronic administration of buspirone or 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT).* PHARMACOL BIOCHEM BEHAV **32**(1) 227–232, 1989.—Cerebrospinal fluid (CSF), collected repeatedly from White Carneau pigeons chronically implanted with guide cannulae located in the lateral ventricles, was analyzed for metabolites of serotonin, dopamine and norepinephrine after acute and chronic administration of buspirone or 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). Following the acute administration of 3.0 mg/kg buspirone, levels of 5-hydroxyindoleacetic acid (5-HIAA) decreased, while increases occurred in the dopamine metabolites homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC). Decreases in 5-HIAA persisted throughout the chronic dosing regimen (36 days), while dopamine metabolites returned to control levels within 8 days. When chronic buspirone was discontinued, levels of 5-HIAA were restored to predrug control levels, while levels of HVA, DOPAC and 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) decreased. All metabolites returned to predrug control levels within one week following buspirone discontinuation except for MHPG, which remained depressed. When the acute effects of buspirone were reexamined, levels of 5-HIAA were again significantly decreased, while HVA and DOPAC levels, as well as those of MHPG, increased significantly. Acute administration of the 5-HT_{1A} receptor ligand 8-OH-DPAT (3.0 mg/kg) resulted in large decreases in 5-HIAA levels that persisted throughout the period of chronic administration. Neither acute nor daily administration of 8-OH-DPAT changed levels of HVA, DOPAC or MHPG. Large increases in 5-HIAA occurred when chronic 8-OH-DPAT was discontinued but declined within one week to control levels. Following a two-week drug-free period, 8-OH-DPAT again caused a significant reduction in 5-HIAA levels. These studies demonstrate that metabolites from pigeon CSF can be reliable and informative indices of drug action over many months of sampling from the same subject. Results demonstrating similar acute and chronic effects of 8-OH-DPAT and buspirone on 5-HIAA reaffirm the importance of serotonin in mediating the anxiolytic effects of buspirone. However, the large rebound in 5-HIAA following cessation of daily 8-OH-DPAT, but not buspirone, suggests differences in the serotonergic mechanisms of these two drugs that may only become apparent, or may be enhanced, following chronic administration. Finally, although clinical reports have shown no indications of withdrawal from buspirone, the decline in noradrenergic activity, as indicated by decreased MHPG levels following chronic buspirone administration, suggests that cessation of buspirone does result in transient neurochemical changes in the noradrenergic system.

Chronic administration Buspirone 8-OH-DPAT Neurochemistry Cerebrospinal fluid (CSF) Pigeon

BUSPIRONE, an azaspirodecanedione that is structurally unrelated to the benzodiazepines and does not bind to benzodiazepine receptors, has been shown to be clinically efficacious in alleviating symptoms of anxiety (13, 18, 29). Buspirone has mixed pharmacological actions and interacts with both dopaminergic (1, 27, 34) and serotonergic (5-HT) systems (17,36). Based on in vitro and in vivo receptor and biochemical experiments, it has been suggested that bus-

[1]Requests for reprints should be addressed to Michael A. Nader, Ph.D., University of Chicago, Department of Psychiatry, Box 411, 5841 South Maryland Avenue, Chicago, IL 60637.

pirone produces its dopaminergic activity as an antagonist at the D_2 receptor (20,21), whereas its serotonergic actions are mediated through the 5-HT_{1A} receptor subtype (26).

In animal studies, buspirone increases food-maintained responding that has been suppressed by punishment (4, 16, 37), an effect characteristic of a number of anxiolytic compounds (3,31). Compared to other species, these increases in punished responding following buspirone administration are particularly robust and reliable in pigeons and are not blocked by the benzodiazepine receptor antagonist Ro 15-1788 (5), by the dopamine agonist apomorphine, or by the dopamine antagonist haloperidol (38), suggesting that buspirone's actions on suppressed responding are not mediated through these neurotransmitter systems. Although it has been suggested that a metabolite of buspirone, 1-pyrimidinylpiperazine (1-PP) may be involved in buspirone's mechanism of action (15), this compound has been shown to exert little behavioral activity in the pigeon and, in fact, is not a buspirone metabolite in this species (5). Recent evidence indicates that buspirone's anticonflict actions may be mediated through the serotonin system. For example, the 5-HT agonist MK-212 attenuated, while the 5-HT antagonist cyproheptadine enhanced buspirone's increases in punished responding of pigeons (39). In addition, 5-HT lesions produced with 5,7-dihydroxytryptamine (5,7-DHT) block buspirone's anticonflict effects in rats (12).

Results from drug-discrimination studies provide further evidence for serotonergic involvement in buspirone's actions by demonstrating that buspirone shares discriminative stimulus effects with the 5-HT_{1A} receptor ligand 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (9, 22, 35). Administration of 8-OH-DPAT, like buspirone, also increases punished responding of pigeons (23,39). Neurochemical analyses from pigeon cerebrospinal fluid (CSF) indicates that both buspirone and 8-OH-DPAT decrease levels of 5-hydroxyindoleacetic acid (5-HIAA), the primary metabolite of serotonin. However, acute doses of 8-OH-DPAT have only minimal effects on the dopamine metabolites homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC), while buspirone increases levels of these metabolites in a dose-dependent manner (23).

In vitro analyses following orally administered buspirone in rats revealed similar dose-dependent increases in HVA and DOPAC without affecting 5-HIAA levels (8). However, 7 days of chronically administered buspirone (20 mg/kg, twice daily, PO) significantly attenuated the increases in both dopamine metabolites, although levels were still elevated above control. A dosing regimen of 25 consecutive days of chronic buspirone did not affect the number of dopamine receptors in the rat striatum (8).

One feature of buspirone that is unique for clinically active antianxiety compounds is that it typically takes approximately 2–3 weeks of chronic administration before it becomes active (19,25). The present study was designed to investigate the effects of daily, long-term administration of buspirone or 8-OH-DPAT on metabolites of seortonin (5-HIAA), dopamine (HVA and DOPAC) and norepinephrine, 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), from cerebrospinal fluid of pigeons with chronic indwelling guide cannulae implanted in the lateral ventricles. Compared to other species, the behavioral effects of buspirone in the pigeon appear to more closely resemble other clinically effective anxiolytics (3), and, therefore, may provide valuable information regarding buspirone's mechanism of action.

METHOD

Subjects

Fifteen adult male White Carneau pigeons, obtained from Palmetto Pigeon Plant, Sumter, SC, served as subjects. All subjects were experimentally naive, prior to the start of the study. Pigeons were housed in individual stainless steel cages with water, crushed grit and Purina Pigeon Checkers available continuously. The colony room was maintained at constant temperature (72 degrees F), humidity (55%) and lighting (0600–2000 hr).

Surgical Procedure and Perfusion Techniques

Pigeons were anesthetized with a mixture of 10 mg/kg pentobarbital and 30 mg/kg ketamine, administered intramuscularly, prior to stereotaxic surgery. After opening the skin over the skull, a small hole was drilled at the following coordinates: AP=+6.8, L=+1.8; stereotaxic center was used as the reference. A guide cannula was attached to a push-pull perfusion assembly (Plastic Products, Roanoke, VA), cut so that the inner tube extended 1.0 mm beyond the tip of the concentric guide cannula. Attached to the push-pull tubes were polyethylene tubing, filled with phosphate-buffered saline solution (PBS) prior to lowering the cannula into the brain. Puncture of the lateral ventricle was indicated when the solution in the tubing dropped precipitously. The guide cannula was anchored to the skull with four stainless steel mounting screws and cranioplastic cement. The guide tube was closed by a threaded "dummy" cap to protect the system from contaminants when experiments were not being conducted. When samples were being collected, the pigeon was placed in a Plexiglas cubicle and the dummy cap was replaced with the push-pull cannula. Cerebrospinal fluid (CSF) was collected using either a Harvard synchronous push-pull perfusion pump, through tubing attached to gas chromatography syringes (1.0 mm Hamilton Syringe, Reno, NV), or a Gilson Minipuls 3 pump, through silicone tubing attached to the push-pull cannula. Both pumps were set to withdraw approximately 1–2 microliters CSF per minute, with samples typically collected over a 10-minute period. The push-pull assembly was modified by cutting the push tube 1.0 mm shorter than the guide cannula. CSF was withdrawn through the "push" side of the cannula, with the "pull" side vented to the atmosphere (7) to minimize pressure changes within the ventricular system. Collected samples were injected into polyethylene tubes and frozen immediately on dry ice. Samples were stored at −70 degrees C until analyzed by high pressure liquid chromatography (HPLC) with electrochemical detection. The procedure for neurochemical assays of dopamine, serotonin and norepinephrine metabolites has been described in detail elsewhere (23).

Chronic Drug Studies

All subjects were sampled once per week for the duration of the study. Sixty minutes prior to sample collection, subjects were injected intramuscularly with either drug or saline and placed in a Plexiglas chamber where they remained for the entire pretreatment interval. Subjects were first sampled one week postsurgery and again for the next two weeks following saline injections. These three data points were averaged and used as drug-free control levels. Chronic drug administration began when pigeons were injected with either 3.0 mg/kg buspirone (N=7) or 3.0 mg/kg 8-OH-DPAT (N=8)

60 minutes prior to sample collection. These doses were selected on the basis of previous studies with acute administration showing marked behavioral (3–5, 38, 39) and neurochemical activity (23) in the pigeon. Drugs were administered 7 days per week, with CSF samples collected on days 1, 8, 15, 22, 29 and 36. Saline was first substituted for drug 60 minutes prior to sample collection on chronic day 43. Saline injections continued throughout the week until pigeons were again sampled following saline. Acute effects of buspirone or 8-OH-DPAT were reexamined following these two control samples. After redetermining the acute effects of either drug, saline was administered for two more sampling sessions.

Data Analysis

Average levels of the metabolites 5-HIAA, HVA, DOPAC and MHPG were calculated under saline and drug conditions. Data are expressed in absolute measures of pg/μl of CSF. Statistical significance was determined by a two-tailed Student's t-test for paired observations, using $p<0.05$ as the level of significance.

RESULTS

Chronic Buspirone

Predrug control levels of 5-HIAA, HVA, DOPAC and MHPG from pigeon CSF are represented by the open bars on the left in Fig. 1. Administration of 3.0 mg/kg buspirone significantly decreased 5-HIAA levels, whereas levels of the dopamine metabolites HVA and DOPAC were significantly increased ($p<0.05$). While MHPG levels were also increased, these did not quite reach the level of statistical significance.

Throughout the chronic phase, 5-HIAA levels remained significantly below control levels except on day 8. Levels of 5-HIAA were below control values in 5 of 6 subjects on this day. However, in one subject, 5-HIAA levels were elevated compared to control. Initial increases in HVA and DOPAC declined during the first week of chronic buspirone and levels of these metabolites were no longer significantly different from controls on day 8. HVA and DOPAC metabolite levels remained largely unchanged across the period of chronic buspirone administration. MHPG levels were somewhat more variable than those of the other metabolites and remained slightly elevated over the time of chronic administration when compared to control.

When saline was substituted after 42 consecutive days of buspirone administration, 5-HIAA levels did not significantly change from levels observed during day 36 of chronic buspirone (Fig. 1, open bars, day 43). Although it did not reach the level of statistical significance, a large decline in HVA was observed, while DOPAC levels were significantly decreased ($p<0.05$) following saline substitution. MHPG levels, although statistically unaffected during the chronic dosing regimen, were significantly decreased when saline was substituted for buspirone ($p<0.05$). This decrease in MHPG remained significantly below predrug control levels one week after discontinuation of chronic buspirone (day 50), while 5-HIAA, HVA and DOPAC returned to pre-chronic control levels and did not differ from those measures.

Following the two-week drug-free period, readministration of buspirone on day 57 again produced large decreases in 5-HIAA levels ($p<0.01$) and increases in HVA ($p<0.01$) and DOPAC ($p<0.05$). In addition, MHPG levels were significantly increased following buspirone ($p<0.05$). The acute

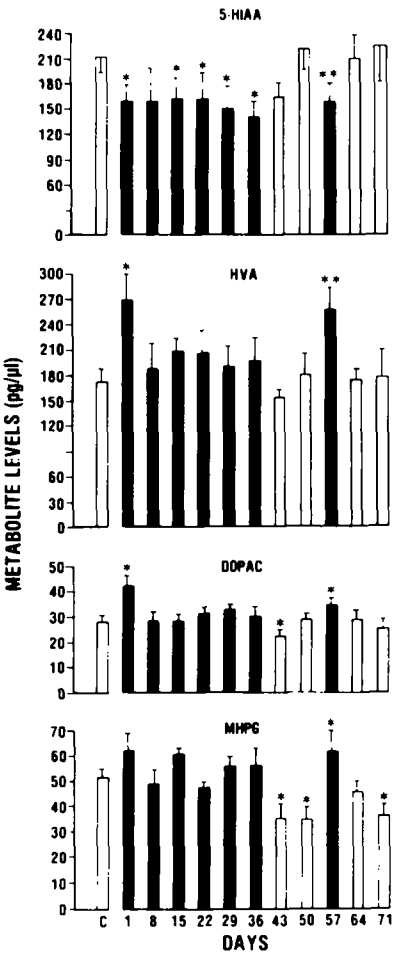

FIG. 1. Neurochemical effects during daily administration of 3.0 mg/kg buspirone on the appearance of 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA), dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) in the cerebrospinal fluid of pigeons. The ordinate represents metabolite levels (pg/μl), while the abscissa represents continuous sampling sessions. Open bars represent saline controls, with the far left bar representing the mean of the first three control samples. Filled bars represent metabolite levels acquired following buspirone administration. Vertical bars represent standard errors of the mean and asterisks indicate statistical significance (*$p<0.05$; **$p<0.01$).

effects of buspirone after chronic administration did not differ from effects obtained on day 1 prior to the period of chronic buspirone. Levels of 5-HIAA, HVA and DOPAC obtained at the end of the study (days 64 and 71) were not significantly different from predrug control levels, while MHPG levels on day 71 were significantly lower than controls obtained at the start of the study ($p<0.017$).

Chronic 8-OH-DPAT

Metabolite levels from pigeon CSF following saline and 3.0 mg/kg 8-OH-DPAT administration are shown in Fig. 2. Levels of 5-HIAA were significantly reduced following

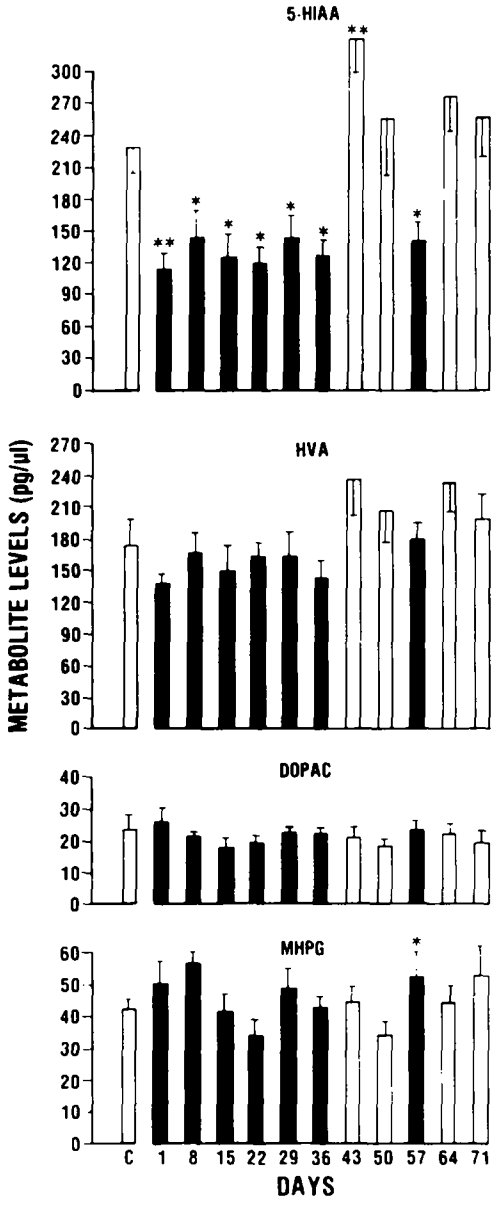

FIG. 2. Neurochemical effects during daily administration of 3.0 mg/kg 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) on the appearance of 5-HIAA, HVA, DOPAC and MHPG levels from pigeon cerebrospinal fluid. Details are as described in Fig. 1.

8-OH-DPAT administration ($p<0.01$). These levels remained significantly below control throughout chronic testing. HVA levels declined slightly following 8-OH-DPAT, but were never significantly different from control during chronic drug administration. DOPAC and MHPG levels were also not significantly different from control across the six weeks of chronic 8-OH-DPAT sampling sessions.

When saline was substituted for 8-OH-DPAT (Fig. 2; day 43), levels of HVA, DOPAC and MHPG remained relatively unchanged compared with levels obtained during day 36 of chronic drug administration. However, discontinuation of 8-OH-DPAT produced significant increases in metabolite levels of 5-HIAA ($p<0.01$), which returned to control levels by the second saline administration session (day 50). HVA levels, although increased, were not significantly different from levels obtained on day 36, nor were they significantly different from predrug controls. Levels of all metabolites were within predrug control levels one week after cessation of daily 8-OH-DPAT administration (day 50). MHPG levels reached their lowest levels on day 50, although the differences, when compared to predrug levels, were not statistically significant.

When the acute effects of 8-OH-DPAT were reexamined (day 57), levels of HVA and DOPAC were again unchanged, while 5-HIAA levels were significantly decreased compared to control levels from day 50 ($p<0.05$). The changes in 5-HIAA levels produced by 3.0 mg/kg 8-OH-DPAT on day 57, following chronic exposure, were not different from those obtained from initial administration of this drug (day 1). However, compared to controls on day 50, MHPG levels were now significantly increased when 8-OH-DPAT was administered following the chronic dosing regimen ($p<0.05$). The increases in MHPG levels on day 57 were not significantly different from the effects observed on day 1 of chronic 8-OH-DPAT administration, suggesting that the significant increases in MHPG seen on day 57 were a result of the postchronic decrease in MHPG control levels. One month after chronic 8-OH-DPAT was terminated (days 64 and 71), all metabolite levels were within predrug control levels.

DISCUSSION

Changes in metabolite levels for serotonin, dopamine and norepinephrine during daily administration of 3.0 mg/kg buspirone or 8-OH-DPAT were examined in pigeons with indwelling guide cannulae chronically implanted in the lateral ventricles. Both buspirone and 8-OH-DPAT decreased levels of 5-HIAA; low 5-HIAA levels persisted throughout the 36 days of chronic drug administration. As has been reported previously, buspirone, but not 8-OH-DPAT, also produced effects on the dopamine system, increasing levels of both HVA and DOPAC (23). Buspirone's action on the dopamine metabolites HVA and DOPAC declined by day 8 when the levels of these metabolites returned to control. Throughout the chronic phase, neither drug appeared to interact significantly with the noradrenergic system.

When saline was substituted after 42 days of chronic drug administration, no significant changes in 5-HIAA were reported following chronic buspirone, whereas large increases in levels of 5-HIAA were seen when 8-OH-DPAT was discontinued. This could indicate some compensatory action in the serotonin system that attenuated the decreases in serotonin levels following 8-OH-DPAT administration. This process may be similar to the behavioral compensatory actions following drug administration that have been attributed to conditioning factors (32). The absence of a similar effect with buspirone following chronic administration may be due to buspirone's mixed actions on both the serotonin and dopamine systems or, as has been suggested previously, because buspirone functions as a partial agonist at presynaptic 5-HT autoreceptors (10). This may also account for the slightly greater decreases in 5-HIAA levels produced by 8-OH-DPAT compared to those of buspirone in the present study. Additionally, however, the different effects of buspirone and 8-OH-DPAT may suggest differences in the mechanisms of action of the two drugs on the 5-HT system

that only become apparent after the cessation of chronic dosing.

While neither drug appeared to alter MHPG levels during daily dosing, a reduction in noradrenergic function became apparent when chronic buspirone administration was discontinued. Whether this decline in norepinephrine metabolism is a result of an interaction with the serotonin or dopamine systems, or attributable solely to noradrenergic involvement, remains to be determined. Evidence for a serotonin-norepinephrine interaction has been reported following destruction of serotonin neurons by 5,7-DHT. After lesions, levels of normetanephrine, a metabolite of norepinephrine (NE) following O-methylation, is significantly decreased in rat cerebral cortex (14), and the amount of NE present in the synaptic cleft is greatly reduced (6). Also, noradrenergic depletion by the neurotoxin N-2-chloroethyl-N-ethyl-2-bromobenzylamine (DSP4) antagonized serotonin-induced analgesia (2). Buspirone, in contrast to most benzodiazepine anxiolytics, increases noradrenergic activity (30) so it is not unexpected that there may be rebound effects after chronic administration. In view of the fact that quantitatively similar decreases in MHPG did not occur after chronic 8-OH-DPAT, these effects after buspirone may also reflect an interaction between dopamine and serotonin as well as more simply, a minimal effect of 8-OH-DPAT on MHPG. Studies of the type conducted here in which buspirone effects are compared with the more dopaminergically inert buspirone analogue gepirone, would be helpful in clarifying these issues. Although these neurochemical changes are in contrast to clinical data with humans or animal data indicating an absence of withdrawal signs upon termination of chronic buspirone administration (11, 28, 33), it does appear that there are neurochemical compensatory changes following chronic administration of either buspirone or 8-OH-DPAT. Whether these changes are of clinical significance remains to be determined. Further, the present data suggest that more sensitive or perhaps different types of behavioral measures may be required to observe the behavioral changes following chronic buspirone that are correlated with the reduction in MHPG.

When the acute effects of either buspirone or 8-OH-DPAT were redetermined after a two-week period, 5-HIAA levels were again significantly decreased by these drugs. There were no differences in 5-HIAA levels between the first administration of buspirone or 8-OH-DPAT (day 1) and the acute effects determined after chronic dosing. Similarly, even though levels of HVA and DOPAC returned to predrug levels during chronic buspirone, when buspirone was again administered following a drug-free period, the increases in HVA and DOPAC returned, suggesting that tolerance was not due to a permanent receptor modification. This is consistent with in vitro data indicating that chronic buspirone administration did not significantly alter the number of dopamine receptors in the rat striatum (8).

In the present study, the fact that the metabolites returned to predrug control levels at the end of chronic administration suggests that daily administration of buspirone or 8-OH-DPAT does not produce permanent changes in serotonin or dopamine levels. Similarities in the behavioral effects of buspirone and 8-OH-DPAT have been attributable to serotonin and, since ventricular CSF 5-HIAA levels have been shown to be a good index of brain serotonin activity (24), studies incorporating pigeon CSF metabolites may lend valuable additional information that could help elucidate buspirone's mechanism of action under both acute and chronic conditions, as well as the actions of other novel anxiolytic drugs.

ACKNOWLEDGEMENTS

We thank Christelle Harrod for the HPLC-ED analysis of the pigeon cerebrospinal fluid, Stephen Ahlers for his assistance on the statistical analyses and Myra Zimmerman for help with the preparation of the manuscript. This work was supported in part by Public Health Service Grant DA-02873.

REFERENCES

1. Algeri, S.; DeLuigi, A.; DeSimoni, M. G.; Imeri, L.; Marconi, M.; Nava, S.; Perego, C.; Sacchetti, G. Multiple and complex effects of buspirone on central dopaminergic system. Pharmacol. Biochem. Behav. 29:823–826; 1988.
2. Archer, T.; Arweström, E.; Minor, B. G.; Persson, M.-L.; Post, C.; Sundström, E.; Jonsson, G. (+)-8-OH-DPAT and 5-MeODMT induced analgesia is antagonised by noradrenaline depletion. Physiol. Behav. 39:95–102; 1987.
3. Barrett, J. E.; Witkin, J. M. Buspirone in animal models of anxiety. In: Tunnicliff, G.; Eison, A. S.; Taylor, D. P., eds. Buspirone: Mechanisms and clinical aspects. New York: Academic Press; in press.
4. Barrett, J. E.; Witkin, J. M.; Mansbach, R. S. Behavioral and pharmacological analysis of the effects of buspirone. Fed. Proc. 43:931; 1984.
5. Barrett, J. E.; Witkin, J. M.; Mansbach, R. S.; Skolnick, P.; Weissman, B. A. Behavioral studies with anxiolytic drugs. III. Antipunishment actions of buspirone in the pigeon do not involve benzodiazepine receptor mechanisms. J. Pharmacol. Exp. Ther. 238:1009–1013; 1986.
6. Brunello, N.; Mocchetti, I.; Volterra, A.; Cuomo, V.; Racagni, G. Serotonergic modulation of cortical rat noradrenergic system in the mechanism of action of antidepressant drugs. Psychopharmacol. Bull. 21:379–384; 1985.
7. Chaouloff, F.; Laude, D.; Guezennec, Y.; Elghozi, J. L. Motor activity increases tryptophan, 5-hydroxyindoleacetic acid, and homovanillic acid in ventricular cerebrospinal fluid of the conscious rat. J. Neurochem. 46:1313–1316; 1986.
8. Cimino, M.; Ponzio, F.; Achilli, G.; Vantini, G.; Perego, C.; Algeri, S.; Garattini, S. Dopaminergic effects of buspirone, a novel anxiolytic agent. Biochem. Pharmacol. 32:1069–1074; 1983.
9. Cunningham, K. A.; Callahan, P. M.; Appel, J. B. Similarities in the stimulus effects of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), buspirone, and TVXQ 7821: Implications for understanding the actions of novel anxiolytics. Soc. Neurosci. Abstr. 11:45; 1985.
10. DeVivo, M.; Maayani, S. Characterization of the 5-hydroxytryptamine$_{1A}$ receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in guinea pig and rat hippocampal membranes. J. Pharmacol. Exp. Ther. 238:248–253; 1986.
11. Eison, M. S. Lack of withdrawal signs of dependence following cessation of treatment or Ro-15,1788 administration to rats chronically treated with buspirone. Neuropsychobiology 16:15–18; 1986.

12. Eison, A. S.; Eison, M. S.; Stanley, M.; Riblet, L. A. Serotonergic mechanisms in the behavioral effects of buspirone and gepirone. Pharmacol. Biochem. Behav. 24:701–707; 1986.
13. Feighner, J. P.; Merideth, C. H.; Hendrickson, G. A. A double-blind comparison of buspirone and diazepam in outpatients with generalized anxiety disorder. J. Clin. Psychiatry 43:103–107; 1982.
14. Ferron, A.; Descarries, L.; Reader, T. A. Altered neuronal responsiveness to biogenic amines in rat cerebral cortex after serotonin denervation or depletion. Brain Res. 231:93–108; 1982.
15. Garattini, S.; Caccia, S.; Mennini, T. Notes on buspirone mechanisms of action. J. Clin. Psychiatry 43:19–22; 1982.
16. Geller, I.; Hartmann, R. J. Effects of buspirone on operant behavior of laboratory rats and cynomologus monkeys. J. Clin. Psychiatry 43:25–32; 1982.
17. Glaser, T.; Traber, J. Buspirone: Action on serotonin receptors in calf hippocampus. Eur. J. Pharmacol. 88:137–138; 1983.
18. Goldberg, H. L.; Finnerty, R. J. The comparative efficacy of buspirone and diazepam in the treatment of anxiety. Am. J. Psychiatry 136:1184–1187; 1979.
19. Jacobson, A. F.; Dominguez, R. A.; Goldstein, B. J.; Steinbrook, R. M. Comparison of buspirone and diazepam in generalized anxiety disorder. Pharmacotherapy 5:290–296; 1985.
20. McMillen, B. A.; Matthews, R. T.; Sanghera, M. K.; Shepard, P. D.; German, D. C. Dopamine receptor antagonism by the novel antianxiety drug, buspirone. J. Neurosci. 3:733–738; 1983.
21. McMillen, B. A.; Mattiace, L. A. Comparative neuropharmacology of buspirone and MJ-13805, a potential anti-anxiety drug. J. Neural Transm. 57:255–265; 1983.
22. Mansbach, R. S.; Barrett, J. E. Discriminative stimulus properties of buspirone in the pigeon. J. Pharmacol. Exp. Ther. 240:364–369; 1987.
23. Mansbach, R. S.; Harrod, C.; Hoffmann, S. M.; Nader, M. A.; Lei, Z.; Witkin, J. M.; Barrett, J. E. Behavioral studies with anxiolytic drugs. V. Behavioral and *in vivo* neurochemical analyses in pigeons of drugs that increase punished responding. J. Pharmacol. Exp. Ther. 246:114–120; 1988.
24. Mignot, E.; Serrano, A.; Laude, D.; Elghozi, J.-L.; Dedek, J.; Scatton, B. Measurement of 5-HIAA levels in ventricular CSF (by LCEC) and in striatum (by *in vivo* voltammetry) during pharmacological modifications of serotonin metabolism in the rat. J. Neural Transm. 62:117–124; 1985.
25. Pecknold, J. C.; Familamiri, P.; Chang, H.; Wilson, R., Alarcia, J.; McClure, D. J. Buspirone: Anxiolytic? Prog. Neuropsychopharmacol. Biol. Psychiatry 9:639–642; 1985.
26. Peroutka, S. J. Selective interaction of novel anxiolytics with 5-hydroxytryptamine$_{1A}$ receptors. Biol. Psychiatry 20:971–979; 1985.
27. Riblet, L. A.; Taylor, D. P.; Eison, M. S.; Stanton, H. C. Pharmacology and neurochemistry of buspirone. J. Clin. Psychiatry 43:11–16; 1982.
28. Rickels, K.; Schweizer, E.; Csanalosi, I.; Case, W. G.; Chung, H. Long-term treatment of anxiety and risk of withdrawal. Arch. Gen. Psychiatry 45:444–450; 1988.
29. Rickels, K.; Weisman, K.; Norstad, N.; Singer, M.; Stoltz, P.; Brown, A.; Danton, J. Buspirone and diazepam in anxiety: A controlled study. J. Clin. Psychiatry 43:81–86; 1982.
30. Sanghera, M. K.; McMillen, B. A.; German, D. C. Buspirone, a nonbenzodiazepine anxiolytic, increases locus coeruleus noradrenergic neuronal activity. Eur. J. Pharmacol. 86:107–119; 1982.
31. Sepinwall, J.; Cook, L. Behavioral pharmacology of antianxiety drugs. In: Iversen, L. L.; Iversen, S. D.; Snyder, S. H., eds. Handbook of psychopharmacology. vol. 13. New York: Plenum Press; 1978:345–393.
32. Siegel, S. A pavlovian conditioning analysis of morphine. In: Krasnegor, N. A., ed. Behavioral tolerance: Research and treatment implications. NIDA Research Monograph 18, U.S. Government Printing Office, (ADM)82–551; 1978:27–53.
33. Taylor, D. P.; Allen, L. E.; Becker, J. A.; Crane, M.; Hyslop, D. K.; Riblet, L. A. Changing concepts of the biochemical action of the anxioselective drug buspirone. Drug Dev. Res. 4:95–108; 1984.
34. Taylor, D. P.; Riblet, L. A.; Stanton, H. C.; Eison, A. S.; Eison, M. S.; Temple, D. L., Jr. Dopamine and antianxiety activity. Pharmacol. Biochem. Behav. 17(Suppl. 1):25–35; 1982.
35. Tricklebank, M. D.; Neill, J.; Kidd, E. J.; Fozard, J. R. Mediation of the discriminative stimulus properties of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) by the putative 5-HT$_{1A}$ receptor. Eur. J. Pharmacol. 133:47–56; 1987.
36. VanderMaelen, C. P.; Wilderman, R. C. Iontophoretic and systemic administration of the non-benzodiazepine anxiolytic drug buspirone causes inhibition of serotonergic dorsal raphe neurons in rats. Fed. Proc. 43:947; 1984.
37. Weissman, B. A.; Barrett, J. E.; Brady, L. S.; Witkin, J. M.; Mendelson, W. B.; Paul, S. M.; Skolnick, P. Behavioral and neurochemical studies on the anticonflict actions of buspirone. Drug Dev. Res. 4:83–93; 1984.
38. Witkin, J. M.; Barrett, J. E. Interaction of buspirone and dopaminergic agents on punished behavior of pigeons. Pharmacol. Biochem. Behav. 24:751–756; 1986.
39. Witkin, J. M.; Mansbach, R. S.; Barrett, J. E.; Bolger, G. T.; Skolnick, P.; Weissman, B. Behavioral studies with anxiolytic drugs. IV. Serotonergic involvement in the effects of buspirone on punished behavior of pigeons. J. Pharmacol. Exp. Ther. 243:970–977; 1987.