

Research Reports

Buspirone, 8-OH-DPAT and ipsapirone: effects on hippocampal cerebellar and sciatic fiber excitability

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The effects of serotonin (5-hydroxytryptamine; 5-HT), and the novel anxiolytics buspirone, 8-OH-DPAT (8-hydroxy-2-[*N,N*-dipropylamino]-tetralin) and ipsapirone (TVXQ 7821, 2-[4-[4-[2-pyrimidinyl]-1-piperazinyl]butyl]-1,2-benzisothiazol-3[²H]one-1,1-dioxide-hydrochloride) on fiber excitability were studied in three axon systems; hippocampal Schaffer collateral fibers, cerebellar parallel fibers, and sciatic nerves. In the hippocampus, application of buspirone, 8-OH-DPAT and ipsapirone resulted in reversible, dose-dependent reductions in the amplitude and conduction velocity of action potentials recorded from presynaptic afferent fibers. Although these agents bind to 5-HT_{1A} receptors, 5-HT application, even at very high (1 mM) concentrations, did not alter axonal responses. This suggests that buspirone, 8-OH-DPAT and ipsapirone exert an action independent of a serotonergic mechanism. Similar effects were observed on the cerebellar parallel fibers although the cerebellum does not have an appreciable number of 5-HT_{1A} receptors. To examine the generalized effects of these agents on nerve excitability, rat sciatic compound action potentials were studied with sucrose gap recordings. Whereas 5-HT, 8-OH-DPAT and ipsapirone had no effects even at high concentrations (1 mM), application of buspirone led to reversible decrement of the responses without appreciable change in membrane potential. These results indicate that buspirone, 8-OH-DPAT and ipsapirone have actions on the excitability of hippocampal and cerebellar neurons independent of serotonergic mechanisms. Moreover, buspirone, but not 8-OH-DPAT or ipsapirone, produces decreased sciatic nerve excitability.

INTRODUCTION

Buspirone is a recently developed non-benzodiazepine anxiolytic drug that has received a great deal of attention from both clinicians and basic scientists. While displaying an efficacy equal to diazepam or chlordiazepoxide in the treatment of anxiety, it lacks the sedation, memory impairment, potentiation of alcohol, and addictive potential associated with the benzodiazepines^{9,18,20}.

Buspirone's precise mechanism of action is unknown. Initial studies suggested an interaction with brain dopamine systems²⁵ as the basis for its anxiolytic properties. This hypothesis was questioned when it was found that an active metabolite of buspirone (1-PP), as well as several other compounds (8-hydroxy-2-[*N,N*-dipropylamino]-tetralin (8-OH-

DPAT), ipsapirone), were effective in animal models of anxiety but lacked significant interaction with dopamine receptors. More recently, radioligand binding studies have shown that buspirone and the novel anxiolytics display potent and selective affinity for the 5-hydroxytryptamine_{1A} (5-HT_{1A}) receptor site^{8,16,18,26}.

Evidence supporting buspirone's proposed role as an agonist at 5-HT_{1A} receptors has come from electrophysiologic studies in rat hippocampus^{2,4,19,20} and dorsal raphe^{24,27,29}. Buspirone weakly mimicks the actions of 5-HT on hippocampal pyramidal cells as well as partially blocks the effects of 5-HT². Application of buspirone results in inhibition of neuronal spike activity; the presence of somatodendritic 5-HT_{1A} autoreceptors as defined by [³H]8-OH-DPAT labeling^{6,11} has thus led to speculation that the novel

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anxiolytics function as agonists or partial agonists at this site.

Although several studies have demonstrated actions of the novel anxiolytics that are mediated via 5-HT receptors, others¹⁷ have presented evidence for electrophysiological actions independent of 5-HT systems. 5-HT has direct actions on the pyramidal cell membrane (rapid hyperpolarization and decreased input resistance^{9,12,22,23}) which result in reduced pyramidal cell spike activity. In contrast, buspirone elicits only a small hyperpolarization and decrease in input resistance². Yet buspirone attenuates both excitatory postsynaptic potentials (EPSPs) and pyramidal cell population spike amplitude evoked by stimulation of afferent fibers in the stratum radiatum^{14,17,20}. Furthermore, examination of presynaptic fiber excitability indicates that buspirone causes diminished fiber excitability (conduction slowing, increased refractory period and impaired ability to follow high frequency stimulation), while 5-HT has no measurable effects on the afferent fibers^{14,17}.

These data raise the possibility that the attenuation of synaptic activation of hippocampal pyramidal cells may be mediated by effects on the afferent fibers. The present study characterizes and compares the effects of 5-HT, buspirone, 8-OH-DPAT and ip-sapirone on the excitability of presynaptic fibers in the hippocampal stratum radiatus. Additionally, the effects of these agents on fiber excitability are examined in fibers not known to involve serotonergic transmission or to contain 5-HT_{1A} receptors — cerebellar parallel fibers and peripheral (sciatic) nerve.

MATERIALS AND METHODS

Hippocampal slice studies

Hippocampal slices were prepared and maintained *in vitro* as follows: female Wistar rats were deeply anesthetized with sodium pentobarbital (70 mg/kg, *i.p.*) and rapidly exsanguinated by carotid section. The dorsal surface of the brain was exposed and a 7 mm block of one hemisphere was removed by making cuts at the midline and at an angle 35° rostral to the coronal plane. The tissue block was immediately placed in cold (4 °C), modified Krebs' solution, glued to the stage of a vibratome with cyanoacrylate and 400 μm slices were cut from the entire block. The hippocampi were dissected free and placed in a submer-

sion-type brain slice chamber. The chamber was continuously perfused with the modified Krebs' solution (3–5 ml/min) and maintained at 34 °C. Sections were allowed to stabilize at least 1 h prior to all experiments. The modified Krebs' solution consisted of the following (in mM): NaCl 124, KCl 3.0, MgCl₂ 2.0, CaCl₂ 2.0, NaHCO₃ 26.0, NaH₂PO₄ 1.3 and dextrose 10.0. The solution was saturated with 95% O₂ and 5% CO₂. All drugs were applied via bath application dissolved in the Krebs' solution.

Drugs being tested were applied at various concentrations (ranging from 1 to 500 μM) in order to assess dose–response characteristics. All drug concentrations were tested at least three times, each on fresh tissue. The test period of 30 min was followed by a wash-out period of 30 min.

Afferent fibers in the CA1 stratum radiatum (Schaffer collaterals and commissural fibers) were orthodromically activated by constant current, cathodal pulses (40 μs duration) delivered through tungsten microelectrodes positioned in the stratum (Fig. 1A). Field potentials elicited by this stimulation were recorded in the dendritic layer of the stratum radiatum with glass microelectrodes filled with 3 M NaCl (DC resistance 5–20 MΩ). These evoked field potentials (Fig. 1B) consist of a short latency large negative response, which represents the action potential of the afferent fibers (the N1 potential). This is followed by a longer duration negativity (N2), corresponding to local excitatory post-synaptic potentials (EPSPs)¹. Often a small positive potential representing distal source currents from synchronous pyramidal population activity is superimposed on the N2 potential. These signals were amplified, digitized with a Nicolet 1174 signal averager and stored on magnetic tape.

Cerebellar slice studies

Rats were prepared as described above, and the bone overlying the caudal brain and brainstem was removed allowing exposure of the cerebellum. The cerebellar peduncles were incised, allowing the cerebellum to be gently dissected free. The tissue was immediately placed in cold (4 °C) modified Krebs' solution, glued to the vibratome stage with cyanoacrylate and 400 μm slices were cut along the longitudinal axis of the folia. The slices were placed in the chamber and subsequently stabilized in a manner identical to that described for hippocampal slices above.

Parallel fibers run the length of each folium and are located in the superficial layers. The fibers were activated by constant current, cathodal pulses (40 μ s duration) delivered through a tungsten microelectrode. Stimulation of the cerebellar surface leads to a well-known field potential that can be recorded along the longitudinal axis of a folium⁷. Standard microelectrodes as described above were used. The early positive-negative-positive (P1-N1-P2) components of the field correspond to the action potential activity of the parallel fibers (Fig. 5A). With these studies of the cerebellar parallel fibers and those involving afferent fibers in the hippocampus, the amplitude and

latency of the Na potential was used as an index of the excitability of the fibers¹³.

Sciatic nerve studies

Female Wistar rats were anesthetized with sodium pentobarbital (70 mg/kg, i.p.), rapidly exsanguinated by a carotid section, and the sciatic nerve was identified and exposed. A 2.0 cm section of nerve was dissected free and placed in oxygenated modified Krebs' solution (as above). Under the dissecting microscope, the nerve was gently desheathed of epineurium and connective tissue, and placed in the sucrose gap recording chamber (Fig. 6A). The gap chamber consisted of 3 compartments each separated by a thin partition of vaseline. The left compartment contained isotonic KCl, the center compartment isotonic sucrose, and the right compartment the test solution (or modified Krebs' solution). All solutions flowed continuously at a rate of 0.5–1 ml/min. The section of nerve in the test compartment was stimulated with Teflon-coated bipolar stainless-steel electrodes. Recordings were obtained with a high-impedance amplifier between the left and right compartments.

Drugs

Drugs were mixed fresh for each experiment in modified Krebs' solution and were applied in the bathing medium. Each drug concentration was tested separately on fresh tissue 3 or more times (1 run per experiment). Drugs were obtained from the following sources: 5-HT (Sigma); buspirone (Bristol-Myers); 8-OH-DPAT (Research Biochemicals); ipsapirone (Troponwerke, Cologne).

RESULTS

Comparison of 5-HT, buspirone, 8-OH-DPAT and ipsapirone on stratum radiatum field potentials

Stratum radiatum stimulation activates non-myelinated afferent fibers which make synaptic contact with pyramidal cell dendrites. A typical field potential recording consists of the afferent fiber action potential (N1), the resulting EPSP (N2), and often a positivity reflecting distal spike activity (Fig. 1B). Application of 5-HT (Fig. 1C; 100 μ m) in concentrations even as high as 1 mM (Fig. 1D) has no measurable effect on this waveform.

By contrast, buspirone, 8-OH-DPAT and ipsapi-

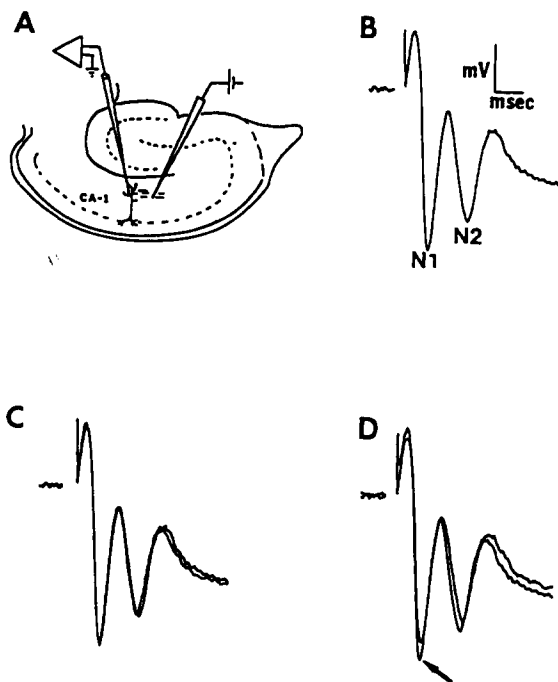


Fig. 1. Effects of 30 min 5-HT application on evoked stratum radiatum field potentials. A: schematic of the in vitro hippocampal slice with placement of stimulating and recording electrodes in the stratum radiatum. B: normal presynaptic response following afferent stimulation, consisting of a large amplitude, short latency potential (N1) which represents action potential currents of the Schaffer collateral afferent fibers. Before returning to baseline, a second negative deflection occurs (N2). This corresponds to sink currents of the EPSP and indicate viable synapses. The N2 may often then be followed by a superimposed positive potential; this represents source currents of distal post-synaptic pyramidal cell spike activity. C: 30 min application of 100 μ m 5-HT. D: 1 mM 5-HT produced either no measurable change in the stratum radiatum field potential, or a small increase in N1 amplitude (arrow).

rone result in reversible, dose-dependent attenuation of the response. Buspirone ($50\ \mu\text{M}$) leads to conduction slowing as evidenced by a small ($50\text{--}70\ \mu\text{s}$) increase in N1 latency (Fig. 2A). This change becomes first apparent approximately 10–20 min after application. The effects of $100\ \mu\text{M}$ buspirone begin within 10–15 min of application with a progressive increase in N1 latency and decreased amplitude (Fig. 2B). Additionally, the EPSP (N2 potential) is markedly attenuated and the positivity reflecting source currents generated from somatic action po-

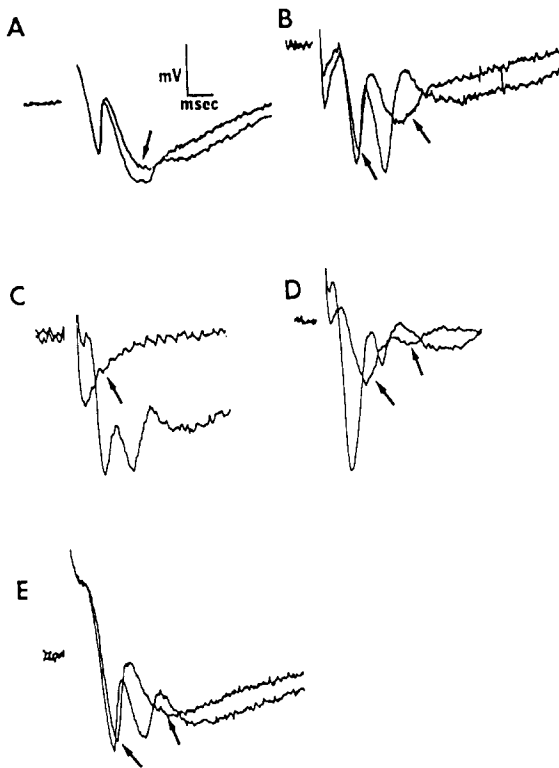


Fig. 2. A: application of $50\ \mu\text{M}$ buspirone for 30 min leads to a gradual increase in N1 latency and a small decrease in the N2 potential (arrow). These effects become apparent at approximately 20 min post-application. B: the effects of $100\ \mu\text{M}$ of buspirone (arrows) begin within 10–15 min of application, with increased N1 latency and decreased amplitude. Note also markedly attenuated N2 (EPSP) and absence of the superimposed positive distal population spike. C: applying $500\ \mu\text{M}$ buspirone produces a rapid (onset 5 min) decrement in both N1 and N2 at 15 min, the response has all but disappeared; partial recovery is seen after prolonged (45 min) wash. D: $100\ \mu\text{M}$ 8-OH-DPAT (arrows) causes rapid (10 min) reduction of N1 amplitude as well as marked slowing of the response. Postsynaptic responses (EPSP, distal population spike) have disappeared. E: $100\ \mu\text{M}$ ipsapirone leads to a gradual increase in N1 latency and amplitude decrement over 30 min, accompanied by reduction and slowing of the N2 responses.

tentials has disappeared. When recordings are made with $500\ \mu\text{M}$ buspirone (Fig. 2C), a faster (onset 5–10 min) decrement in amplitude and latency results, progressing to complete response loss at 15 min. Whereas full recovery occurs with the lower doses after 15 min. of wash with drug-free solution, only partial recovery is seen after prolonged (45 min) wash when the high dose ($500\ \mu\text{M}$) is used.

The effects of 8-OH-DPAT and ipsapirone were qualitatively similar to those observed with buspirone, the major differences being the dose required to achieve a given degree of change. Whereas $100\ \mu\text{M}$ of 8-OH-DPAT (Fig. 2D) led to relatively rapid reduction of response amplitude and conduction slowing leading ultimately to nearly complete block (similar to $500\ \mu\text{M}$ buspirone), $100\ \mu\text{M}$ ipsapirone behaved like low-dose buspirone with a more gradual onset of conduction slowing and relatively small reduction in amplitude. The dose–response characteristics of each drug is illustrated in Fig. 3 by rank order of potency, 8-OH-DPAT > buspirone > ipsapirone.

Double stimulation experiments were carried out in drug-free solutions and compared with results obtained using buspirone $100\ \mu\text{M}$, 8-OH-DPAT $100\ \mu\text{M}$ and ipsapirone $100\ \mu\text{M}$ in order to assess the effects of these agents on axonal excitability (Fig. 4). In normal electrolyte solution, paired stimuli delivered 2 msec apart results in impaired conduction of the second (test) response; the N1 potential was delayed and amplitude diminished because of the fiber's relative refractory period. On the other hand, interstimulus

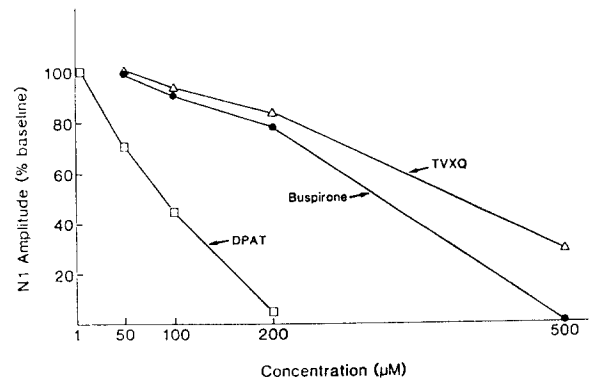


Fig. 3. Dose–response effects of buspirone, 8-OH-DPAT, and ipsapirone (TVXQ), on the amplitude of presynaptic hippocampal field potentials. Each data point represents the mean of at least 3 separate experiments per drug concentration (1 experiment per slice). The amplitude of the field potential is expressed as the percentage of pre-drug baseline.

intervals of 5 to 150 msec resulted in a decreased latency of the conditioned response, indicative of the supernormal period.

Application of buspirone $100\ \mu\text{M}$ (Fig. 4) and the other agents appears to functionally extend the relative refractory period from 2 ms to approximately 20 ms. Furthermore, the period of supernormal conduction is nearly abolished. This prolongation of the refractory period, accompanied by reductions in response amplitude and conduction velocity, suggests an activity-dependent decrease in fiber excitability.

Effects of 5-HT, buspirone and 8-OH-DPAT on cerebellar parallel fibers

Stimulation of the cerebellar surface leads to a well-defined field potential (Fig. 5A) corresponding to the action potential activity of the parallel fibers. Application of 5-HT, even in high concentration (1 mM), has no measurable effect on either amplitude or latency of the response (Fig. 5E,F). By contrast, buspirone $100\ \mu\text{M}$ results in progressive reduction in amplitude and increased latency beginning approximately 10–15 min after application (Fig. 5B). This effect is fully reversed after a wash-out period of 30 min. Similar to the effects seen in the hippocampus, a higher dose of buspirone ($500\ \mu\text{M}$) leads to progressive conduction block over 10 min until the response is nearly completely abolished (Fig. 5C). Two

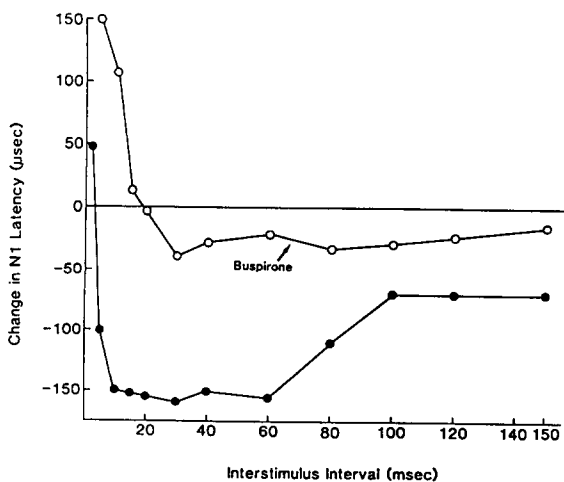


Fig. 4. Representative comparison ($n = 6$) of normal saline and buspirone ($100\ \mu\text{M}$, 30 min) on fiber excitability (N1 latency) during double stimulation. Change in N1 latency of the conditioned response plotted against interstimulus interval. Note that buspirone attenuates the decrease in latency (i.e. the supernormal period) produced by double stimulation.

hundred μM 8-OH-DPAT is seen to be equipotent to $500\ \mu\text{M}$ buspirone in its ability to cause conduction blockade (Fig. 5D).

Effects of 5-HT, buspirone, 8-OH-DPAT and ipsapirone on sciatic nerve compound action potentials

Sciatic nerves, 2.0–2.5 cm in length were placed in a sucrose gap recording chamber as represented schematically in Fig. 6A. A typical compound action potential with normal Krebs' solution through the test compartment is shown in Fig. 6B. Even at concentrations up to 1 mM, 5-HT, 8-OH-DPAT and ipsapirone had no measurable effects on the responses after 30 minutes of administration (Fig. 6D–F). However, buspirone (1 mM) led to progressive decrement of the action potential (Fig. 6C) with recovery to near-baseline amplitude following wash with drug-free solution. Examination of dose–response characteristics found buspirone to have no effect until doses of $100\ \mu\text{M}$ were used, and significant changes in amplitude (50% or greater decrement) were observed only at the dose of 1 mM. No appreciable changes in

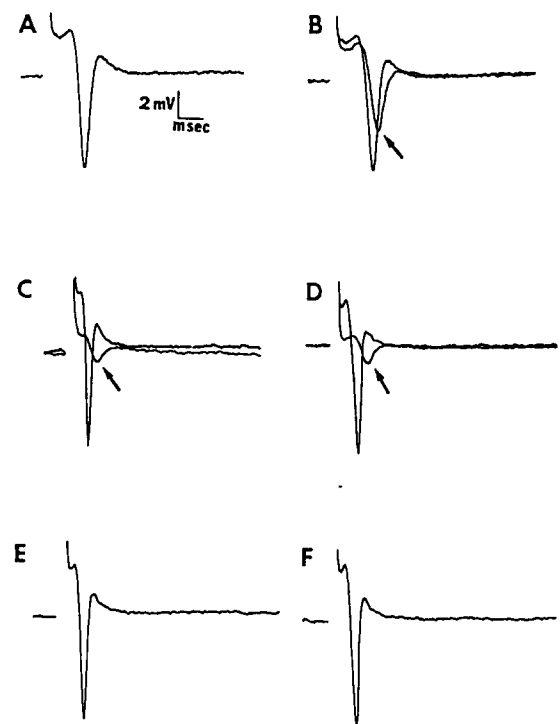


Fig. 5. Effects of drug application on cerebellar parallel fiber potentials. A: normal electrolyte solution. B: $100\ \mu\text{M}$ buspirone at 30 min (arrow). C: $500\ \mu\text{M}$ buspirone at 15 min (arrow). D: $200\ \mu\text{M}$ 8-OH-DPAT at 15 min (arrow). E, F: response before and after 1 mM 5-HT.

membrane potential were observed, even at high doses.

DISCUSSION

The major finding of the present study is that buspirone leads to a reversible, progressive and dose-dependent decrease in fiber excitability that appears to be independent of an interaction with serotonergic transmission. Comparison of buspirone's effects with those of 5-HT were made in hippocampal stratum radiatum afferents, in cerebellar parallel fibers, and in sciatic nerve. While high doses of 5-HT consistently have no measurable effects on the recorded responses, buspirone application leads to reversible, dose-dependent reductions in response amplitude

and conduction velocity (increased response latency). Furthermore, that these effects are due to diminished excitability is supported by paired stimulation experiments in which buspirone, 8-OH-DPAT and ipsapirone resulted in prolongation of the relative refractory period and reduction of the supernormal period.

Previous electrophysiologic studies addressing the effects of buspirone and the other novel anxiolytics (8-OH-DPAT, ipsapirone) on the hippocampal population spike and on pyramidal cell membrane properties have yielded conflicting results. For example, Andrade and Nicoll² reported that buspirone administration elicited a small hyperpolarization of pyramidal neurons, accompanied by a decrease in input resistance; these effects were smaller than those in-

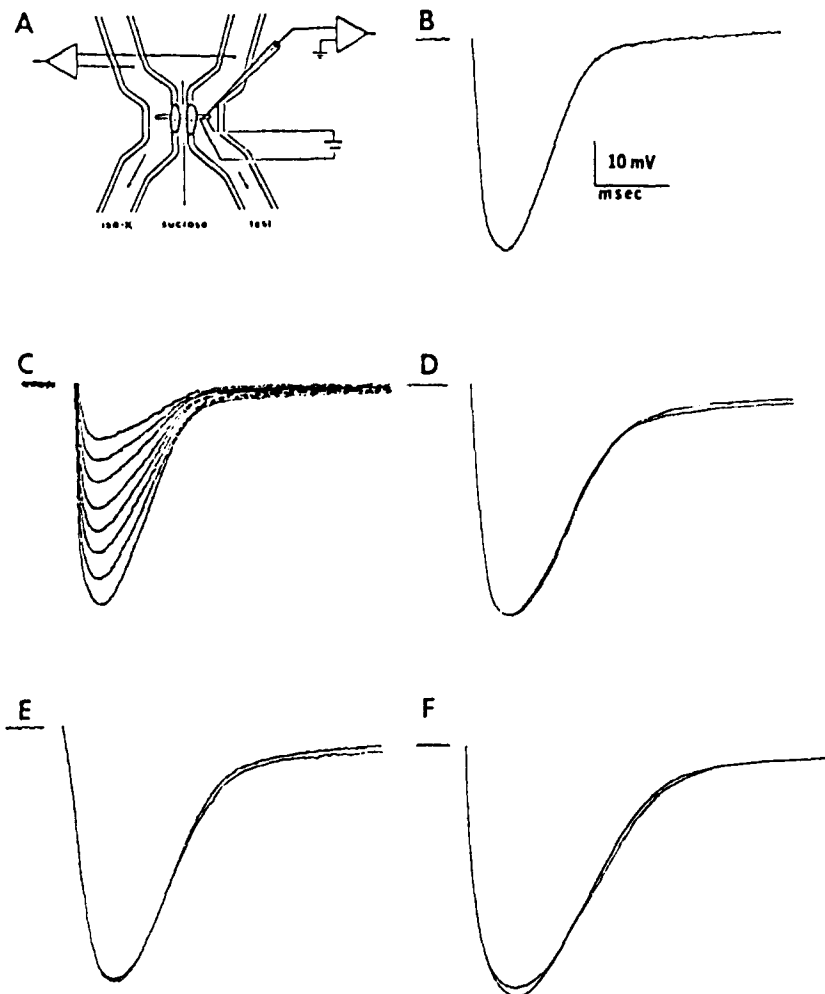


Fig. 6. Effects of 30 min drug application on sciatic nerve compound action potentials. A: schematic of sucrose gap. B: normal electrocyte solution. C: 1 mM buspirone. D: 1 mM 8-OH-DPAT. E: 1 mM ipsapirone. F: 1 mM 5-HT.

duced by serotonin. Furthermore, a dose-dependent antagonism of serotonin's effects by buspirone led them to postulate that buspirone functions as a weak, partial agonist at the 5-HT_{1A} site. Similarly, others^{3,4,19,20} have suggested a 5-HT_{1A} receptor-mediated mechanism for the inhibitory effects of buspirone and ipsapirone on the pyramidal population spike. Studies in rat dorsal raphe also find that these drugs inhibit neuronal discharge, and the presence of somatodendritic 5-HT_{1A} autoreceptors on raphe neurons has helped reinforce the concept that the novel anxiolytics function as 5-HT_{1A} agonists^{21,24,27,29}.

Recent studies^{14,17} showed that 5-HT markedly hyperpolarized CA1 pyramidal cells, yet has no demonstrable presynaptic effects. By contrast, buspirone has little direct effect on pyramidal cell membrane potential or resistance, yet resulted in diminished EPSP activity. Preliminary findings indicating a presynaptic site of buspirone action were substantiated and extended in this study. Hippocampal stratum radiatum field potentials were consistently attenuated after buspirone application. A spectrum of effects was seen, ranging from small increases in response latency at low (10–50 μm) doses, to more pronounced conduction slowing with reduced amplitude (100 μm), and ultimately to complete conduction block and loss of response at high (500 μm) doses. This is in contrast to 5-HT, which had no effect on the response even at millimolar concentrations.

The possibility of a non 5-HT_{1A}-mediated mechanism for at least one of buspirone's actions was further investigated by examining responses in fiber systems known to contain few, if any, 5-HT receptors⁵. Field potentials recorded from cerebellar parallel fibers were remarkably similar to those from the stratum radiatum in their responses to 5-HT and buspirone. Once again, 5-HT had no demonstrable effect whereas buspirone displayed a reversible dose-dependent reduction in fiber excitability as manifested by progressively increased latencies and amplitude reductions leading ultimately to nearly complete response abolition as higher doses (300–500 μm) were approached.

Further support for a non-serotonergic mechanism mediating these presynaptic actions was obtained when effects on peripheral (sciatic) nerve were observed in the sucrose gap chamber. Application of buspirone (100 μm) led to a small reversible decre-

ment in the compound action potential; at higher doses (1 mM), a marked decrement of the response (to less than 33% of baseline) occurred, which reversed to near baseline levels after wash-out. This effect was not accompanied by any appreciable change in membrane potential. Although relatively high doses were required, this is likely the result of peripheral nerve diffusion characteristics. Despite nerve desheathing prior to study, some residual connective tissue may be present. Epineurium, perineurium and endoneurium, as well as myelin, may serve as barriers to passive diffusion of the drug.

The other 5-HT_{1A} novel anxiolytics examined, 8-OH-DPAT and ipsapirone were similar to buspirone in their effects on hippocampal stratum radiatum and cerebellar parallel fiber field potentials (i.e., reversible conduction slowing and amplitude reduction). The only differences were in potency; the same magnitude decrease in fiber excitability caused by a given dose of buspirone could be achieved with lower doses of 8-OH-DPAT and yet require a higher dose of ipsapirone. Thus, in terms of rank-ordering potency, 8-OH-DPAT > buspirone > ipsapirone >>> 5-HT, which produced no attenuation of fiber excitability.

The observation that the most potent agent (8-OH-DPAT) in the hippocampus and cerebellum had no effect on peripheral nerve serves to undermine the argument that buspirone's action on sciatic nerve fiber is simply a local anesthetic effect. Mediated by Na⁺ channel blockade, an anesthetic effect would be expected to equally affect channels in CNS tissue (i.e. hippocampus, cerebellum) as well as those in peripheral nerve. 8-OH-DPAT's potency in central tissues, and lack thereof on peripheral tissue suggests that a single anesthetic mechanism of action cannot adequately explain the phenomenon of reduced fiber excitability in those tissues so affected. Rather, that only buspirone displayed an effect on peripheral nerve may relate to other factors, such as lipophilicity and other diffusion characteristics.

It may be argued that the doses employed in this study far exceed the individual KD for each agent as demonstrated by 5-HT_{1A} binding studies. Indeed, the data presented herein demonstrates that at least one of the novel anxiolytics' actions (attenuation of synaptic activation of hippocampal pyramidal cells) may not be mediated by the 5-HT_{1A} receptor, as has been previously stated^{3,4,19,20}. These findings may or may

not have relevance to clinical therapy in achieving anxiolysis, or for that matter, to the sequelae of drug overdosage. However, the claim that all of the novel anxiolytics' actions are mediated via the 5-HT_{1A} receptor, especially those involving synaptic activation of hippocampal pyramidal cells, should be revised in light of our findings.

In summary, buspirone is a newly developed non-benzodiazepine drug effective in the treatment of anxiety. It has prompted much work on its mechanism of action using a variety of techniques including radioligand binding, biochemical and behavioral studies. The bulk of these, as well as other physiologic studies, support the proposal that buspirone' actions are mediated via the 5-HT_{1A} receptor. This

study, however, presents evidence that buspirone, 8-OH-DPAT and ipsapirone also exert a presynaptic effect that is independent of their affinity for serotonin receptors.

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