identical in sign. (ii) The behavior of the type III cells could also be attributed to a more generalized lateral inhibitory network consisting of an array of type I cells, all of which have neighboring receptive fields. In this case, the synaptic connections of neighboring type I cells onto type III cells should be opposite in sign.

From this preliminary study, as well as from detailed anatomical studies (*18*), many analogies can be drawn between the retina of the visual system and the PLLL of the electrosensory system. Functionally, both the ganglion cells of the retina and the type III cells of the PLLL are designed to respond maximally to local changes in the stimulus. Local contrast is accentuated, whereas uniform illumination or large-field amplitude modulations have little effect on the discharges of a ganglion cell or a type III cell, respectively.

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4. *Eigenmannia virescens* (10 to 13 cm long) and two species of *Sternopygus—S. macrurus*, and an unidentified species characterized by a golden line running the length of the tail—(15 to 20 cm long) were studied. Fish were acclimatized to water conditions of *p*H 5 to 7, temperature 26° to 28°C, and resistivity of 10 kilohm-cm for at least 4 days before the experiments.
5. Hoffmann-La Roche Co. provided a sample of this drug.
6. The experimental set-up is illustrated in figure 1a of W. Heiligenberg, C. Baker, and J. Matsubara, [*J. Comp. Physiol.* **127**, 267 (1978)]. The artificial EOD signals, which were not phase-locked to the animal's pacemaker, seem to be sufficient for these studies for three reasons. (i) No known anatomical connections (efferent copy of the EOD) exist between the pacemaker and electrosensory processing areas (for example, posterior lateral line lobe or torus semicircularis) for *Sternopygus, Eigenmannia*, and *Hypopomus* (W. Heiligenberg, T. Finger, J. Matsubara, C. Carr, in preparation). In addition, these types of free-running artificial EOD signals are sufficient to drive behaviors such as the JAR in *Eigenmannia*, a closely related gymnotiform [Heiligenberg *et al.* (1978)]. (ii) The stimulus-field geometry produced by the stomach-tail electrode arrangement resembles that of the fish's natural EOD, as demonstrated by mapping isopotentials. However, the waveform of *S1* differs from that of the natural EOD in that it lacks the higher harmonic spectral frequencies. The fish may use these harmonic components of its natural EOD in texture (for example, capacitive) discrimination. (iii) The general features of electrolocation, such as encoding the type of object (conductive or insulative) are identical when using either *S1* or a natural EOD. This was observed by comparing the results from this study with results from studies that used the natural EOD's of *Apteronotus* (*7*).
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8. Since the objects were the same size, the mechanoreceptive cues were nearly identical, whereas, because of the differences in conductivity between the aluminum and Plexiglas, the electrical cues were not. Thus, the mechanoreceptive responses would be identical but the electroreceptive responses would be different. Moreover, there is no electroreceptive response to moving objects if the EOD substitute, *S1*, is removed.
9. Fish were blindfolded with a piece of black electrical tape fitted over the eyes and tied securely around the head.
10. K. Frank and M. Becker, in *Physical Techniques in Biological Research*, W. L. Nastuk, Ed. (Academic Press, New York, 1964), vol. 5, part A, p. 23.
11. For horseradish peroxidase (HRP) injections, the recording electrode was replaced with an HRP-filled glass pipette and lowered to the depth of the recording site.
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13. J. Bastian and W. Heiligenberg, *J. Comp. Physiol.* **136**, 135 (1980).
14. For square-wave amplitude-modulated *S1*'s, types I and II show adapting excitatory responses at the onset or offset, respectively, of the modulation.
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16. The approximate center of the cell's receptive field can be identified by observing the location of the moving object during the maximum firing of the cell. "Local" jamming stimuli were then produced in this area. This was achieved by applying the voltage for the jamming signal through the left carbon rod electrode and a monopolar electrode placed 1 mm from the left side of the fish. The voltage was then decreased until the minimum voltage necessary for the cell to respond was attained (that is, until lowering the voltage any more produced no beat-related response from the cell). Keeping this minimum voltage constant, the monopolar electrode (which was always kept 1 mm from the body surface) was then moved systematically along the length of the fish until the cell's receptive field was defined.
17. P. Enger and T. Szabo [*J. Neurophysiol.* **28**, 800 (1965)] found directionally sensitive neurons in the PLLL of *Apteronotus*. These cells may be similar to type III cells in *Sternopygus*.
18. L. Maler, E. Sas, J. Rogers, in preparation.
19. Supported by NIMH grant PHSMH-2614904 and NSF grant BNS76-20761 to W.H. I thank W. Heiligenberg, T. Bullock, T. Platt, C. Baker, B. Kristan, B. Partridge, and two anonymous referees for their advice and criticisms during this study.

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Interaction Between Purine and Benzodiazepine: Inosine Reverses Diazepam-Induced Stimulation of Mouse Exploratory Behavior

Abstract. *Inosine, 2-deoxyinosine, and 2-deoxyguanosine completely reversed the increase in exploratory activity elicited in mice by diazepam. The inhibition of exploratory behavior by purines occurred at doses that when given alone have no effect on exploratory behavior. 7-Methylinosine, which does not bind to the brain benzodiazepine binding site in vitro, had no effect on the diazepam-induced increase in exploratory behavior. Behavioral effects produced by various combinations of inosine and diazepam indicate that the interaction between purine and benzodiazepine is antagonistic and support the hypothesis that the naturally occurring purines function in anxiety-related behaviors that respond to benzodiazepine treatment.*

The recent discovery of pharmacologically relevant, high-affinity, stereospecific binding sites for the benzodiazepines in the central nervous system (*1*) has prompted studies on the possible physiological significance of these sites and attempts at isolating endogenous ligands (*2*). Several naturally occurring inhibitors of the binding of ^{3}H-labeled diazepam have been isolated from mammalian brain and proposed as endogenous ligands (*3*). Our studies have focused on the purines inosine and hypoxanthine and on the structurally related 2-deoxypurines (*4*). Although these compounds are relatively weak competitive inhibitors of [^{3}H]diazepam binding in vitro, they appear to exist in the brain in high concentrations (*5*) that increase severalfold when brain slices are subjected to depolarizing stimuli (*6*).

The major actions of the benzodiazepines include anticonvulsant, muscle relaxant, and anxiety-reducing effects (*7*). A putative endogenous ligand must demonstrate pharmacological, neurophysiological, and behavioral properties similar to those of the benzodiazepines. Large doses of purines antagonize pentylenetetrazole-induced seizures in mice in a dose-dependent manner (*8*). Inosine applied by microiontophoresis or pressure injection to cultured mouse spinal cord neurons elicited a rapidly desensitizing excitatory response that showed cross-desensitization with benzodiazepines and a nondesensitizing inhibitory response that was blocked by benzodiazepines (*9*). Inosine antagonized the γ-aminobutyric acid (GABA)-mimetic action of diazepam in a model system in which electrical stimulation of the globus pallidus caused head turning in rats (*10*). These lines of evidence support the view that purines have a functional role in benzodiazepine-mediated actions. Since the most specific and clinically applicable property of the benzodiazepines is their anxiety-reducing effect, a putative endogenous ligand for the brain benzodiazepine binding site should provide some measure of anxiolytic action. We have developed a simple, automated, one-parameter test for the behavioral effects of benzodiazepines in mice (*11*) and now report that the purines completely block the behavioral changes produced by diazepam at doses which by themselves do not affect these behaviors.

The test depends on the natural tendency of mice to explore a novel environment, but to avoid a brightly lighted open

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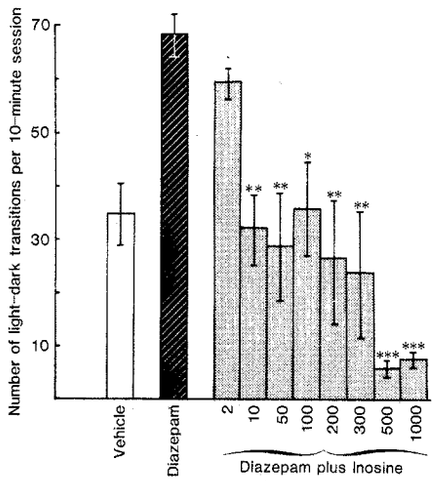

Fig. 1. Inosine reverses the diazepam-induced increase in mouse exploratory behavior. Naïve mice were treated intraperitoneally with diazepam (2 mg/kg) or vehicle 30 minutes before testing and by saline or inosine (2 to 1000 mg/kg) 10 minutes before testing. Numbers below bars indicate dose of inosine in milligrams per kilogram. During a 10-minute test session, the frequency with which an individual mouse crossed the border between a brightly lighted open field and a dark enclosed area was measured. Inosine at doses of 10 to 300 mg/kg completely blocked the increase in activity by diazepam, and sedation was noted at higher doses. Values are expressed as means ± standard errors (S.E.) for six mice in each dosage group; $^{*}P < .05$; $^{**}P < .01$; and $^{***}P < .005$, in comparison with diazepam plus saline.

field. The testing apparatus (*11*) records the number of transitions made by a mouse between a highly illuminated open-field compartment and a dark, enclosed compartment. When benzodiazepines are administered, the number of such transitions is doubled. Although increased exploratory activity in a novel environment is only inferentially related to an antianxiety effect, the data on dose-response relations, rank-order potencies, and pharmacological specificity of benzodiazepines in our exploration experiments (*11, 12*) are similar to findings based on the more intricate animal anxiety models in current use (*13*).

Naïve male Swiss-Webster mice (20 to 25 g) were given an intraperitoneal dose of diazepam (Hoffmann-LaRoche; 2 mg/kg in 2 percent ethyl alcohol and 4 percent propylene glycol, in phosphate-buffered saline at *p*H 7.2), 30 minutes before testing. Ten minutes before testing, inosine, 2-deoxyinosine, 2-deoxyguanosine, 7-methylinosine (Sigma; in phosphate-buffered saline at *p*H 7.2), or saline alone was administered intraperitoneally, at doses ranging from 2 to 1000 mg/kg.

Inosine in doses of 10 to 300 mg/kg reversed the diazepam-induced increase in the number of transitions between compartments (Fig. 1). 2-Deoxyinosine and 2-deoxyguanosine at doses of 50 to 200 mg/kg also reversed the effect of diazepam (*14*). These doses were below the sedative range of inosine (500 to 1000 mg/kg), which significantly reduced behavioral activity when administered either alone or with diazepam (*15*). No significant effect on the number of transitions was found when these purines were administered alone in the dose range 2 to 300 mg/kg (*16*). Administration of 7-methylinosine, which does not inhibit diazepam binding in brain tissue in vitro (*17*), had no effect on the diazepam-induced increase in transitions (*18*), an indication that the purines are not influencing behavior through nonspecific effects.

Since large doses of purines produce sedative effects, and large doses of benzodiazepines have a sedative-hypnotic action (*13*), we examined the possibility that the purines interact synergistically with diazepam to produce a pharmacological summation of the sedative effects of the two drugs. To test this possibility, we administered various doses of inosine with various doses of diazepam. If inosine and diazepam act synergistically, a smaller dose of the purine plus a larger dose of diazepam would produce the same effect as a larger dose of purine plus a smaller dose of diazepam. Alternatively, if inosine and diazepam are antagonistic, larger doses of purine would be necessary to reverse larger doses of diazepam. The results of this experiment indicate an antagonistic relationship between inosine and diazepam (Fig. 2); at 5 mg/kg diazepam, more inosine was required to block the diazepam-induced increase in mouse exploratory activity than at 2 mg/kg or 0.5 mg/kg.

The mechanism by which the various purines antagonize the benzodiazepine-induced increase in exploratory behavior is unclear. Although relatively high concentrations of purine have been shown to competitively inhibit [^{3}H]diazepam binding to benzodiazepine receptors in vitro, it is unlikely that such concentrations are achieved in vivo at the doses used in the present study (10 to 300 mg/kg, intraperitoneally). The interaction between purine and benzodiazepine may occur directly on the benzodiazepine-GABA-linked recognition site (*9, 19, 20*) or its related chloride ionophore (*21*), rather than on a separate benzodiazepine binding site.

Even though the behavior model employed in this study is only theoretically related to human anxiety, it does appear

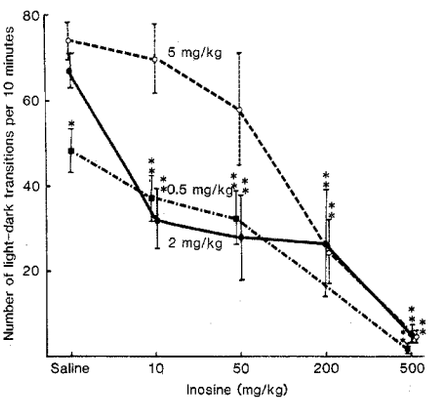

Fig. 2. Antagonistic interactions between diazepam and inosine during exploratory behavior in mice. Varying doses of inosine and diazepam were administered as described in Fig. 1 to test the alternate explanations of synergistic versus antagonistic interactions of purines with benzodiazepines. Doses of diazepam were (——) 2 mg/kg, (- - -) 5 mg/kg, and (—·—·—) 0.5 mg/kg. Larger doses of inosine were required to block the effects of larger doses of diazepam, evidence that the interaction is pharmacologically antagonistic. Each point represents the mean ± S.E. for six mice. $^{*}P < .05$; $^{**}P < .01$, in comparison with diazepam (2 mg/kg) plus saline.

to reflect benzodiazepine activity with as much pharmacological specificity as other animal behavior models in current use (*13*). These experiments suggest that the naturally occurring purines, essential for many biological functions, may play a role in mediating behaviors that are sensitive to the anxiety-reducing actions of the benzodiazepines.

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14. The number of transitions per 10-minute session for diazepam (2 mg/kg) plus various doses of 2-deoxyinosine were: 50 mg/kg, 71.8 ± 9.7; 100 mg/kg, 43.0 ± 9.3 ($P < .05$); and 200 mg/kg, 40.0 ± 12.8 ($P < .05$). For diazepam (2 mg/kg) plus 2-deoxyguanosine, the values were: 50 mg/kg, 46.5 ± 6.1 ($P < .05$); 100 mg/kg, 50.0 ± 6.5 ($P < .05$); and 200 mg/kg, 11.0 ± 6.3 ($P < .01$). P values were determined by comparison with diazepam (2 mg/kg) plus saline.
15. The number of transitions per 10-minute session were: saline, 35.0 ± 5.7; inosine (500 mg/kg), 5.8 ± 1.6 ($P < .005$); and inosine (1000 mg/kg), 7.5 ± 1.5 ($P < .005$).
16. None of the values of transitions per 10-minute session were significantly different from values for saline controls for the following drug doses: inosine 2, 10, 50, 100, 200, and 300 mg/kg; 2-deoxyinosine 50, 100, and 200 mg/kg; 2-deoxyguanosine 50, 100, and 200 mg/kg; and 7-methylinosine 50, 100, and 200 mg/kg.
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Tyrosine Administration Decreases Vulnerability to Ventricular Fibrillation in the Normal Canine Heart

Abstract. *Intravenous infusion of tyrosine (1, 2, or 4 milligrams per kilogram) for 20 to 30 minutes caused dose-dependent increases in the ventricular fibrillation threshold in normal dogs. Administration of valine, a neutral amino acid that competes with tyrosine for uptake at the blood-brain barrier, in a dose equimolar to the most effective dose of tyrosine, slightly decreased the ventricular fibrillation threshold when given alone and significantly blocked elevation of the ventricular fibrillation threshold after tyrosine infusion. Hence, tyrosine, presumably acting in the central nervous system, can protect against certain ventricular arrhythmias.*

The sympathetic nervous system influences cardiac susceptibility to ventricular arrhythmias (*1*). Augmented sympathetic activity, whether elicited by electrical stimulation of the hypothalamus (*2*) or of the stellate ganglia (*3*), predisposes the heart to diverse arrhythmias. Conversely, reduction of sympathetic neural outflow, achieved either surgically (*4*) or pharmacologically (*5*), protects against arrhythmias. Cabot *et al.* (*6*) demonstrated that the raphe nucleus inhibits sympathetic outflow in the pigeon. There is evidence that treatments that increase the release or postsynaptic effects of serotonin, the transmitter of raphe nucleus neurons (*7*), diminish cardiovascular sympathetic outflow (*8*). Blatt *et al.* (*9*) showed that agents that produce an increase in brain serotonin protect the heart against ventricular fibrillation. Rabinowitz and Lown (*10*) found that administration of L-tryptophan, precursor to serotonin, decreases cardiac vulnerability to ventricular fibrillation (VF), probably by increasing brain serotonin release (*11*) and thereby reducing sympathetic neural outflow.

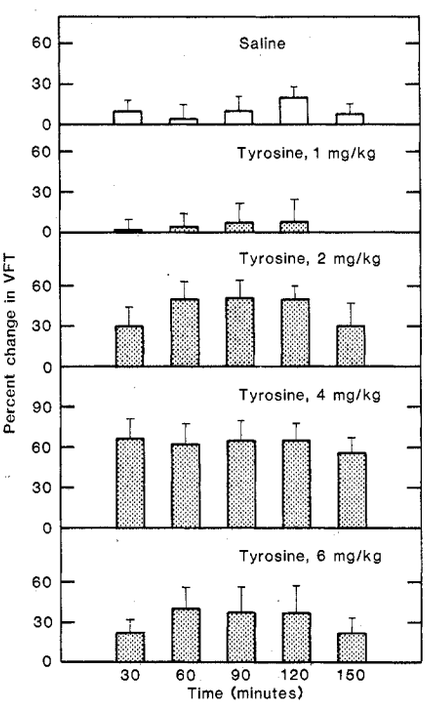

Fig. 1. Effect of tyrosine on ventricular fibrillation threshold (*VFT*). Control VF threshold's were obtained for each dog. The dogs then received intravenous doses of tyrosine (1 mg/kg, $N = 5$; 2 mg/kg, $N = 6$; 4 mg/kg, $N = 6$; or 6 mg/kg, $N = 6$) or vehicle (saline, $N = 8$), and the VF threshold was determined at 30-minute intervals. Percent changes in VF threshold after tyrosine or saline administration are graphed as means and standard errors of the means.

Enhanced central catecholaminergic activity can also diminish sympathetic outflow (*12*). Drugs such as clonidine and α-methyldopa, which presumably act by stimulating α-noradrenergic receptors in the brainstem, decrease blood pressure in hypertensive animals and humans (*13*); moreover, clonidine also decreases vulnerability to VF (*14*) and suppresses digitalis-induced arrhythmias (*15*).

Norepinephrine synthesis (*16*), and probably release (*17*), in the brain can be accelerated by administering its precursor, L-tyrosine, thereby augmenting saturation of the rate-limiting biosynthetic enzyme tyrosine hydroxylase. Tyrosine administration increases brain levels of the norepinephrine metabolite methoxyhydroxyphenylglycol sulfate and decreases blood pressure in spontaneously hypertensive rats (*18*). We hypothesized that administration of tyrosine to healthy dogs, by increasing central catecholaminergic activity and thereby diminishing sympathetic neural outflow to the heart, would raise the threshold of vulnerability and protect against VF.

At least 4 days after arriving from the supplier, healthy mongrel dogs of either sex, weighing 9 to 22 kg, were anesthetized with α-chloralose (100 mg/kg) with additional drug (50 mg/kg) administered as needed to maintain a constant level of anesthesia. Experiments were initiated between 0830 and 0930 hours, at least 30 minutes after induction of anesthesia. The animals were ventilated with a mixture of room air and 40 percent oxygen so that arterial oxygen tension was about 100 mm-Hg. Arterial pH was maintained

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