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Inosine May Be an Endogenous Ligand for Benzodiazepine Receptors on Cultured Spinal Neurons

Abstract. *Mouse spinal neurons grown in tissue culture were used to study the membrane effects of the benzodiazepine flurazepam and the naturally occurring purine nucleoside inosine, which competes for benzodiazepine receptor sites in the central nervous system. Application of inosine elicited two types of transmitter-like membrane effects: a rapidly desensitizing excitatory response and a nondesensitizing inhibitory response. Flurazepam produced a similar excitatory response which showed cross-desensitization with the purine excitation. Flurazepam also blocked the inhibitory inosine response. The results provide electrophysiological evidence that an endogenous purine can activate two different conductances on spinal neurons and that flurazepam can activate one of the conductances and antagonize the other.*

Benzodiazepines are a commonly used class of drugs with a broad spectrum of application as tranquilizers, anticonvulsants, muscle relaxants, and sedatives (*1*). These therapeutic effects are presumably mediated in part through actions on the central nervous system (CNS) since high-affinity, stereospecific, and saturable binding sites for benzodiazepines have been identified throughout the CNS both in vitro (*2*, *3*) and in vivo (*4*). Several substances isolated from the CNS compete with tritiated benzodiazepines for CNS receptor sites (*5*). These endogenous substances have been identified as the purine hypoxanthine and its nucleoside inosine (*6*), both of which are synthesized de novo in neuronal tissue (*7*). In addition to their function in nucleic acid synthesis, purines can be released from nerve tissue (*8*, *9*) and alter the excitability of central (*10*) and peripheral neurons and smooth muscle (*11*), results suggesting that these compounds may mediate intercellular communication in the nervous system (*9*–*11*). We have used cultured spinal neurons to examine the membrane actions of the benzodiazepine flurazepam and the purine inosine and report that (i) these substances produce rapidly desensitizing excitatory responses that cross-desensitize with each other, and (ii) inosine inhibits excitability by increasing membrane conductance, an effect that is blocked by flurazepam. The results provide evidence for two transmitter-like effects of inosine and suggest that flurazepam can act as an agonist at one site and antagonist at the other.

Neurons were dissociated from spinal cords of 13-day-old fetal mice and grown in tissue culture (*12*, *13*). Intracellular recordings were made on the modified

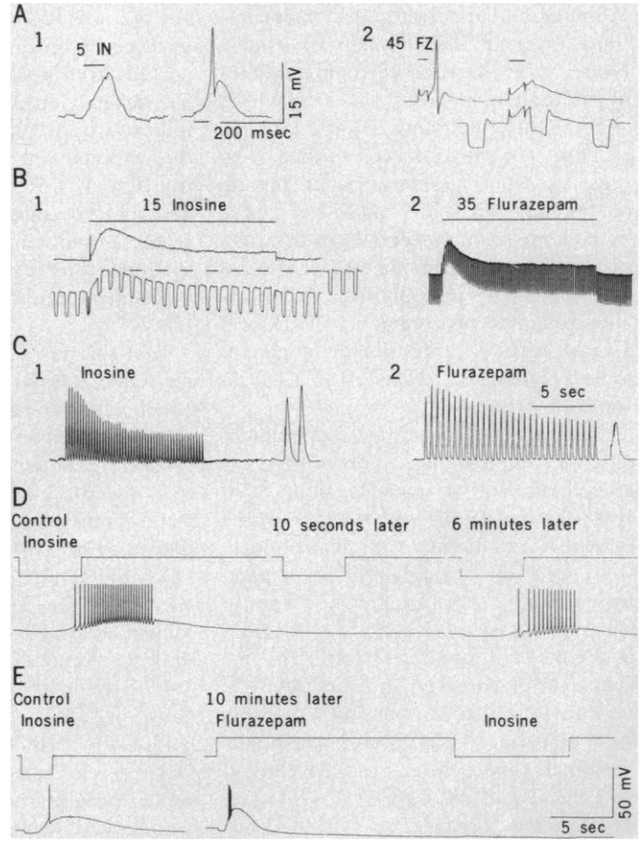

Fig. 1. Comparison of excitatory responses to inosine (*IN*) and flurazepam (*FZ*) on cultured spinal neurons. Pen recorder traces of potassium acetate recordings from eight cells are shown. Bars above traces in (A to C) and Fig. 2 indicate iontophoretic drug application periods, with numbers above bars giving iontophoretic currents in nanoamperes. Voltage calibration bar in (A1) refers to (A) to (C) and time calibration bar in (C2) refers to (B) and (C). (A1) Depolarizing responses to inosine at −62 mV (left) and −55 mV (right) are shown, the latter sufficient to generate a spike whose amplitude is attenuated by the frequency response of the pen recorder. (A2) Flurazepam responses recorded on another cell show excitation at −48 mV (left) and little change in conductance at −55 mV (as reflected in the amplitude of the voltage response to −0.5-nA hyperpolarizing pulses). (B) In sustained applications of inosine (B1) and flurazepam (B2) to two different cells, desensitization of both voltage and conductance changes is shown, the latter assessed with constant −0.4-nA (B1) and −0.5-nA (B2) pulses. Membrane conductance at depolarized level is less than that during inosine application (inset in B1). (C) Repetitive applications of inosine (C1) at 175 nA in 8-msec pulses (four per second) and of FZ (C2) at 150 nA in 40-msec pulses (two per second) on two cells show initial potentiation of response amplitude followed by desensitization during both trains. Complete recovery within 10 seconds is shown in (C1). [Recording speed for last two responses in (C1) and final response in (C2) was five times faster.] (D) Application of inosine by pressure (monitored above voltage trace) from a pipette containing 10 μM inosine causes a brief excitation which completely desensitizes membrane to an inosine application 10 seconds later. Partial recovery is evident 6 minutes later. (E) Cross-desensitization between FZ and inosine is shown. Pressure-applied inosine briefly excites a spinal cord neuron (*Control*); 10 minutes later sustained pressure application of FZ from a 10-μM pipette causes an excitatory response which rapidly desensitizes. The excitatory response to inosine is then completely abolished. The inosine response recovers 10 minutes after termination of FZ application. Membrane potentials (in millivolts): (B1), −60; (B2), −62; (C1), −56; (C2), −60; and (D) and (E), −61.

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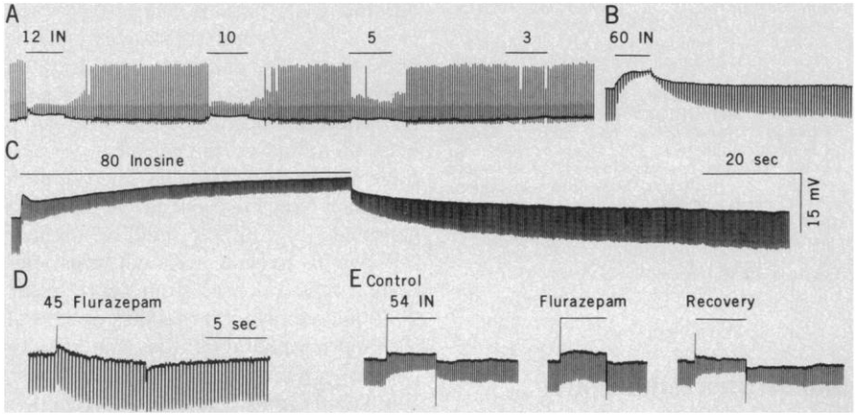

Fig. 2. Inhibitory response to inosine (*IN*) blocked by flurazepam. Pen recorder traces of potassium acetate recordings from five cultured spinal neurons are shown. (A) Inosine depresses excitability in a dose-dependent, reversible manner; excitability was assessed using suprathreshold constant-current pulses. Variability in subthreshold voltage depolarizations reflects variable contribution of "active" responses to the depolarizations. (B) Inosine markedly increases membrane conductance of another cell, as reflected in the depression of the voltage responses to constant-current hyperpolarizing pulses during inosine application. (C) Sustained iontophoresis of inosine initially evokes the rapidly desensitizing excitatory response and then the inhibitory response, marked by an increase in membrane conductance. The latter does not desensitize during prolonged application. The sudden step at end of iontophoresis is caused by a coupling artifact. (D) Flurazepam increases membrane potential and input resistance of another cell. (E) Flurazepam (40 nA) attenuates inosine-induced increase in membrane conductance at the inversion potential of the inosine response. Membrane potentials (in millivolts): (A), −55; (B), −65; (C), −60; (D), −40; and (E), −58. Constant-current pulses (in nanoamperes): (A), +0.8; (B), −0.5; (C), −0.6; (D), −0.5; and (E), −0.6.

stage of an inverted phase microscope at 26°C with micropipettes filled with 3*M* potassium chloride or 4*M* potassium acetate. Magnesium ion (10 m*M*) was added to the bathing medium to suppress ongoing synaptic activity and allow clearer examination of membrane responses. Flurazepam (FZ) (Hoffmann-La Roche, Nutley, New Jersey) was applied either by iontophoresis from an extracellular micropipette containing 200 m*M* FZ (*p*H 3.5) placed close to the cell surface or by pressure from an extracellular pipette containing 10 μM FZ (dissolved in the bathing medium) placed 50 μm from the cell surface. Inosine (Sigma, St. Louis) was applied by iontophoresis and pressure from pipettes containing 40 m*M* and 10 μM inosine, respectively. Hypoxanthine (Sigma, St. Louis) was used in some experiments.

Application of inosine caused a rapidly desensitizing depolarization which excited cells and a nondesensitizing increase in membrane conductance which inhibited excitability. Similar effects were seen with iontophoretically applied hypoxanthine. The excitatory response was rapid in onset (Fig. 1, A1), dose-dependent, and rapid in offset. The response was observed in 37 of 69 cells tested with either iontophoresis or pressure ejection. An excitatory response similar in time course was also elicited with FZ (Fig. 1, A2 and E) (*14–16*). The depolarizing responses to sustained application of both purines and FZ were associated with an increase in membrane conductance (Fig. 1B). Sustained application of either these purines (Fig. 1, B1) or FZ (Fig. 1, B2 and E) revealed a rapid and complete desensitization of the voltage and accompanying conductance changes of both responses. Closely repeated, brief pulses of either purines or FZ resulted in a transient potentiation, followed by a successive depression of the response amplitude relative to control (Fig. 1C). Recovery from a partially desensitized state was usually apparent within 10 seconds (Fig. 1, C1), although recovery after complete desensitization sometimes required 5 to 6 minutes (Fig. 1D).

That the purines and FZ may engage the same site in activating membrane conductance is suggested by the cross-desensitization exhibited by the two responses. Sustained pressure application of a desensitizing amount of FZ completely prevented the depolarizing response to pressure-ejected inosine (Fig. 1E). Conversely, sustained pressure application of a desensitizing amount of inosine blocked FZ-evoked responses (not shown). Similar cross-desensitizations were seen with iontophoretic applications.

Two other, different responses to iontophoresis of inosine and FZ were observed on cultured spinal neurons. The inosine-mediated response consisted of a dose-dependent, reversible increase in membrane conductance sufficient to inhibit excitability (Fig. 2, A and B), the latter being assessed with constant-current, suprathreshold depolarizing pulses. This response was observed on 18 of 65 cells studied. The increase in conductance was always slower in onset when compared with the rapidly depolarizing event and showed little evidence of desensitization (Fig. 2C). Both responses could be elicited in succession on the same cell (Fig. 2C). The inversion potential of this response was similar to that of the putative amino acid transmitter γ-aminobutyric acid (GABA) recorded from the same cells (*17*); this suggests that the conductance activated by the purines is to Cl^- ions, like that activated by GABA (*13*). The FZ-mediated response consisted of a dose-dependent, reversible increase in membrane resistance (Fig. 2, D and E). The inversion potential of the associated voltage response was also similar to that evoked by inosine on the same cells (*17*), which suggests that the observed effects are due to a decrease in Cl^- conductance. This response was observed in 17 of 45 cells examined and was present in the absence of exogenously applied purine. The increase in membrane conductance in response to inosine was antagonized by FZ in a dose-dependent, reversible manner. For example, in Fig. 2E the peak membrane conductance during the inosine response in the absence of FZ averaged 175 percent of base-line membrane conductance, but peak conductance in the presence of FZ was only 108 percent of base line.

These observations show that the purine inosine can alter neuronal excitability in a manner analogous to neurotransmitter action, that is, by activating membrane conductances independent of membrane voltage. The rapidly desensitizing nature of the excitatory response would effectively limit both the intensity and duration of the excitatory signal. Such evanescent excitatory responses have not been reported in the intact CNS for any endogenous or exogenous agonist, but have been observed in molluscan ganglia with acetylcholine, dopamine, and apomorphine (*18*). The similar properties of the FZ-induced excitation and its cross-desensitization with the inosine response suggest that this putative purine receptor may be one site at which these substances might compete for binding (*19*). Although FZ can transiently excite neurons in our experiments, this is unlikely to occur during therapeutic administration of the drug, which involves an increase in drug concentration

throughout the CNS at a rate much slower than can be attained by using either iontophoresis or pressure. Rather, therapeutic application of the drug would be expected to attenuate naturally occurring purine-mediated excitation through cross-desensitization. Another site at which purines and benzodiazepines might interact is the putative purine receptor associated with inhibition of excitability. Therapeutic administration of a benzodiazepine might antagonize these purine-mediated inhibitory events.

Benzodiazepines can modulate the GABA-mediated inhibition of CNS neurons, which suggests that this interaction might account for some of the therapeutic actions of these drugs (*20*). Since GABA does not compete with benzodiazepines for the same receptor site in vitro (*3*), these drugs do not appear to be interacting directly with GABA receptors. The data reported here show that a benzodiazepine can function as an agonist at one putative purine receptor site and as an antagonist at another. These observations, coupled with the fact that specific purines can displace benzodiazepines in binding studies in vivo (*6*) and in culture (*21*), suggest that benzodiazepines interact directly with two classes of purine receptor. The interactions between benzodiazepines and purines, and between benzodiazepines and GABA, and their relationship to the therapeutic actions of the benzodiazepines require further investigation (*22*).

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Target Velocity Signals of Visual Tracking in Vermal Purkinje Cells of the Monkey

Abstract. *Discharges of Purkinje cells were recorded from the vermis, lobules VI and VII, of a monkey trained to track a visual target. When the monkey tracked a sinusoidally oscillating target, cellular activity changed in phase with the velocity signal of the eye movement. When the monkey fixated a stationary point, almost identical modulation in activity occurred, reflecting the velocity signal of the motion of the retinal image of the target. The data suggest that the vermis participates in the control of smooth pursuit eye movements by providing the oculomotor system with the actual target velocity information which is the sum of eye velocity and retinal image velocity signals.*

The maintenance of images on the fovea is a prerequisite for proper visual function and is the responsibility of the pursuit eye movement system. Images of moving objects of interest, having first been brought into the region of maximal visual acuity by saccadic eye movements, are sustained on the fovea by pursuit eye movements. Of the parameters characterizing target motion, velocity is important for tracking (*1*). Since a smooth pursuit eye movement is the result of an attempt to match the angular velocity of the eye movement to the velocity of the target, information concerning target velocity must be available to the pursuit eye movement system. In the presence of free eye and head movements, however, absolute target velocity is not directly available, and its determination depends on information concerning the relative motions of (i) target

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