*Pharmacology Biochemistry & Behavior*, Vol. 24, pp. 121–125, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

# Benzodiazepine-Like Effects of Inosine on Punished Behavior of Pigeons[1]

JEFFREY M. WITKIN AND JAMES E. BARRETT

*Department of Psychiatry, Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799*

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WITKIN, J. M. AND J. E. BARRETT. *Benzodiazepine-like effects of inosine on punished behavior of pigeons.* PHARMACOL BIOCHEM BEHAV **24**(1) 121–125, 1986.—Behavioral effects of the putative endogenous benzodiazepine receptor ligand, inosine, were studied alone and in combination with the benzodiazepine antagonist Ro 15-1788. Keypeck responses were maintained by food under a multiple fixed-interval 3-min, fixed-interval 3-min schedule of food delivery. Under the multiple schedule, the first response after 3 min produced food in the presence of either white (no punishment) or red keylights and, in addition, each 30th response produced a brief electric shock (punishment) when the keylight was red. Inosine increased the low rates of punished responding (10–100 mg/kg IM) and the higher rates of unpunished responding (30 mg/kg). The benzodiazepine antagonist Ro 15-1788 (0.03 mg/kg, IM) antagonized the rate-increasing effects of inosine but had no effect when given alone. Combinations of inosine (30 mg/kg) with higher doses of Ro 15-1788 (0.1–1 mg/kg) decreased responding much like Ro 15-1788 alone. The marked rate-decreasing effects of 1000 mg/kg inosine were not affected by concurrent administration of Ro 15-1788 (0.01–1 mg/kg). The behavioral effects of inosine alone resembled effects of benzodiazepines but not those of benzodiazepine antagonists. The response rate-increasing effects of inosine may be due to its benzodiazepine receptor binding properties, whereas the rate decreases produced by higher doses of inosine appear to be unrelated to benzodiazepine receptors.

Inosine Ro 15-1788 Punished behavior Antagonism Benzodiazepine receptors Pigeons

IDENTIFICATION of saturable, high-affinity, stereoselective receptors in the central nervous system for benzodiazepines [4,15] led to a search for possible endogenous ligands. Such compounds may be physiological regulators of states related to anxiety. The purinergic compound, inosine, was one of the first to be identified [1, 16, 20]. Inosine was found to bind to brain benzodiazepine receptors with affinity in the millimolar range [1, 13, 19]. In some experimental preparations, inosine appears to behave like a benzodiazepine receptor agonist. Pentylenetetrazol-induced seizure latencies are increased by inosine [21] and cross-desensitization to flurazepam is exhibited on mouse spinal neurons in culture [12]. Studied under different conditions, inosine exhibits benzodiazepine-antagonist properties: the increase in exploratory activity of mice [6] and the increased food consumption of rats [11] produced by diazepam are reversed by inosine. Furthermore, flurazepam antagonizes inhibitory effects of inosine on mouse spinal neurons [12]. In the current study, the potential sedative-hypnotic/anxiolytic actions of inosine were evaluated. The specific benzodiazepine receptor antagonist Ro 15-1788 [3, 19, 25] was used to investigate the involvement of benzodiazepine receptors in the behavioral effects of inosine.

Behavior suppressed by response-produced electric shock delivery (punished behavior, sometimes referred to as conflict behavior) is a sensitive and selective baseline to evaluate the potential sedative-hypnotic or anxiolytic potential of compounds. Punished behavior is increased by benzodiazepines, barbiturates and a variety of other drugs with sedative-hypnotic and anxiolytic activity [2, 5, 14, 24]. Similar effects are not obtained with neuroleptics, opiates, or antidepressants nor are the increases in punished behavior typically found after administration of drugs like the amphetamines which normally produce large increases in comparable low rates of unpunished behavior [2, 5, 14].

## METHOD

### *Subjects*

Four adult male white carneaux pigeons were maintained at 80% (420–430 g) of their free-feeding body weights. They were housed in individual cages with continuous access to water and oyster shell grit. The cages were located within a temperature- and humidity-controlled vivarium with a 12 hr light-dark cycle. Three of the pigeons had been studied previously in an experiment in which effects of midazolam and Ro 15-1788 were administered [25]. The fourth pigeon was experimentally-naive prior to the current study.

### *Behavioral Procedure*

Responding was maintained under a multiple fixed-interval (FI) 3-min, fixed-interval 3-min schedule of food

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[2]Requests for reprints should be sent to present address: Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

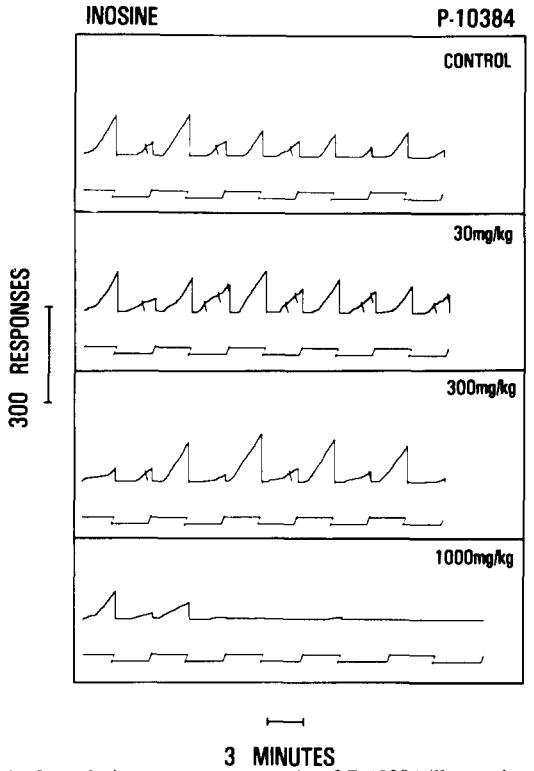


FIG. 1. Cumulative response records of P-10384 illustrating representative control performance under the multiple FI 3-min (no punishment) FI 3-min (with punishment) schedule. Also shown are effects of various doses of inosine showing its rate-increasing effects on punished and unpunished responding at 30 mg/kg, the mixed rate-decreasing and rate-increasing effects of 300 mg/kg, and the pronounced suppression of responding at 1000 mg/kg. The response pen was incremented with each response and was reset at the time of food delivery or automatically if a response was not emitted within 60 sec of the elapse of the 3-min interval. Angular deflections of the response pen occurred during shock delivery. The lower tracing in each panel is displaced downward during the component in which punishment was in effect. The recording paper did not move during the 15-sec timeout period which separated schedule components.

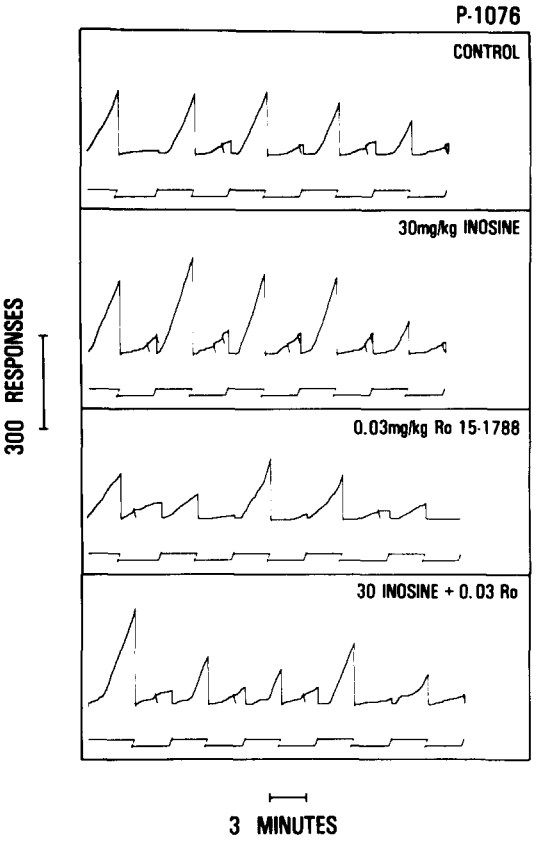


FIG. 2. Cumulative response records of P-1076 illustrating representative control performance under the multiple FI 3-min (no punishment) FI 3-min (with punishment) schedule. Effects of a rate-increasing dose of inosine (30 mg/kg) are also shown alone and in combination with 0.03 mg/kg Ro 15-1788. Although this dose of Ro 15-1788 was without effect when given alone, it reversed the effects of inosine. Recording details as in Fig. 1.

presentation. Responses during one FI component produced electric shock according to a fixed-ratio 30 schedule. Under the multiple schedule, the first response after 3 min in the presence of a white keylight produced food (no punishment); in the presence of a red keylight the first response after 3 min produced food, and, in addition, every 30th response produced shock (punishment). A 15-sec timeout, during which the keylights were extinguished and responding had no scheduled consequences, separated each FI cycle. If a response did not occur within 1 min of the lapse of the FI, timeout was initiated and food was not presented. The experimentally-naive pigeon was initially trained to peck the response key , (cf. [7]) and responses were then maintained under the multiple FI 3-min, FI 3-min schedule. The punishment contingency was added only after responding had stabilized. Experimental sessions consisted of ten FI cycles starting with the white keylight (no punishment); the color of the lights alternated throughout the session. Several weeks of training under this baseline resulted in stable rates of re-

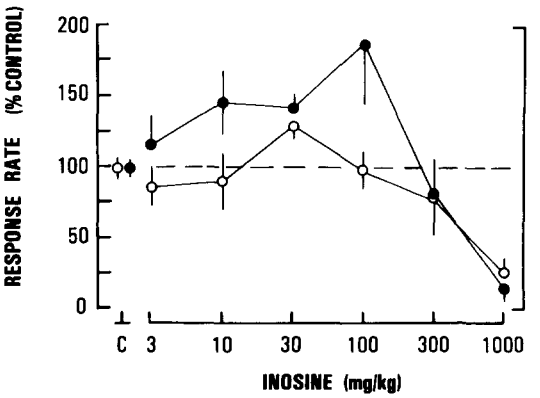


FIG. 3. Effects of inosine on punished (filled circles) and unpunished (open circles) responding (N=4). Means±S.E.M. are shown. Points above C represent control values (N=9). Control rates of unpunished responding ranged between 0.59±0.03 to 0.92±0.11 responses per sec and control rates of punished responding ranged across animals between 0.19±0.02 to 0.26±0.02 responses per sec. Moderate doses of inosine increased both punished and unpunished responding in each of the animals tested.

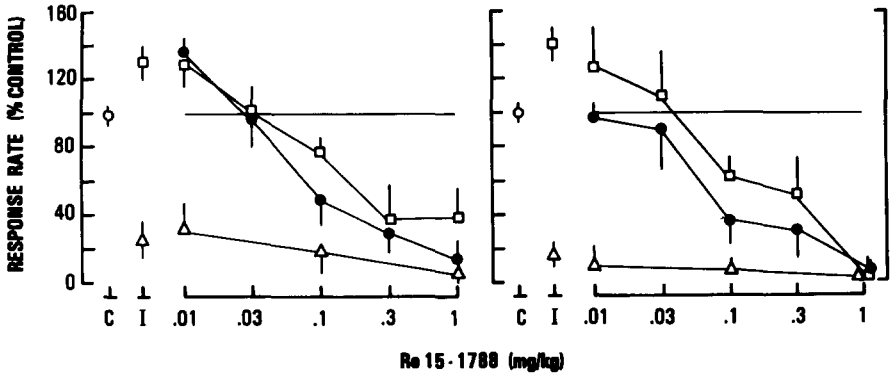


FIG. 4. Effects of 30 mg/kg inosine (squares) and 1000 mg/kg inosine (triangles) alone (points above I) and in combination with Ro 15-1788 (N=4). Effects on unpunished responding are shown in the left panel and the effects on punished responding are presented in the right panel. Effects of Ro 15-1788 alone are represented by the filled circles. Means±S.E.M. are shown. Points above C represent control values (N=15). Control rates of unpunished responding ranged from 0.63±0.04 to 0.87±0.02 responses per sec and control rates of punished responding ranged across birds from 0.14±0.001 to 0.28±0.02 responses per sec. Ro 15-1788 reversed the rate-increasing effects of 30 mg/kg inosine but did not affect the rate decreases produced by 1000 mg/kg inosine.

sponding which were used to assess the effects of inosine and Ro 15-1788.

*Drugs*

Ro 15-1788 (generously donated by Hoffman-LaRoche, Inc., Basel, Switzerland), was prepared in fine suspension with sterile water and Tween 80 (1 drop/5 ml). Inosine (Aldrich Chemical Co., Milwaukee, WI) was dissolved in 0.9% NaCl to a concentration of 150 mg/ml. Intramuscular injections (1.0 ml/kg) were given 60 sec prior to experimental sessions. Inosine, Ro 15-1788 and combinations of the two were studied in a mixed order after effects of inosine and Ro 15-1788 were initially determined. Each dose was studied separately on at least two occasions in each bird and drug combinations were also generally studied in duplicate. Drug doses are expressed as the total base. Drugs were administered no more than twice weekly, on Tuesdays and Fridays, with Thursday's sessions serving as non-injection control sessions.

*Data Analysis*

Rates of responding in each component of the multiple schedule were obtained by dividing total responses during the FI by the total session time elapsed within the component. Drug effects on response rate were compared to rates of responding during vehicle and non-injection control sessions for each bird individually. Drug vehicles were without effect and were therefore averaged with rates obtained on non-injection control sessions. Composite dose-effect functions were derived by averaging changes in individual performances across subjects. Drug effects were considered significant if the mean deviated by at least 2 S.E.M. from the control mean or from the effects of a drug alone.

## RESULTS

Under control conditions, rates of punished responding were at least 68% lower than unpunished response levels. Temporal patterns of punished and unpunished responding were, however, quite comparable; performance was characterized by a period of little or no responding at the beginning of the interval followed by a relatively constant rate of responding until food delivery (Figs. 1 and 2, top panels).

Inosine produced dose-dependent increases in punished responding (Fig. 3, solid symbols) which were maximal at 100 mg/kg. Rates of unpunished responding were increased to 129% of control levels after 30 mg/kg inosine, but were not appreciably affected by other doses from 30 to 300 mg/kg (Fig. 3, open symbols).

The rate-increasing effects of 30 mg/kg inosine are shown in the cumulative response records of Figs. 1 and 2. Rates of punished and unpunished responding were clearly elevated above control levels and the pause in responding at the beginning of each interval was decreased. Rates of shock presentation in the punishment component were increased 2.4 times that prevailing under control conditions. Increases in responding were evident within the first 3 min of the session and generally persisted throughout the 30 min session. Inosine (300 mg/kg) decreased responding early in the session, and unpunished responding was increased subsequently (Fig. 1). The decreases in both punished and unpunished responding after 1000 mg/kg (Fig. 3) were also evident within the first few minutes of the session, and responding was almost completely eliminated for the last 20 min (Fig. 1).

The benzodiazepine antagonist Ro 15-1788 had no effect on the rate-increases produced by 30 mg/kg inosine at 0.01 mg/kg Ro 15-1788 which increased unpunished responding when given alone (Fig. 4). The increases in both punished and unpunished responding were reversed by 0.03 Ro 15-1788 which was without effect when given separately. This antagonism of the effects of 30 mg/kg inosine is shown in the dose-response functions of Fig. 4 and in the cumulative response records of Fig. 2. Higher doses of Ro 15-1788 decreased punished and unpunished responding when given alone or in combination with 30 mg/kg inosine. The pronounced rate-decreasing effects of 1000 mg/kg inosine were not reversed by Ro 15-1788 from 0.01 to 1 mg/kg (Fig. 4, triangles).

## DISCUSSION

Effects of inosine on punished and unpunished responding were comparable to effects of benzodiazepines, barbiturates and other sedative-hypnotic, anxiolytic agents. All of

these drugs increase punished responding at doses which often have little effect on rates of unpunished responding [2, 5, 14, 24]. Although less potent than benzodiazepines under similar experimental conditions, inosine increased punished behavior across a wide range of doses and with comparable efficacy to that of benzodiazepines [2, 14, 25]. The lack of benzodiazepine-like pharmacological activity of inosine in other experimental preparations [16,22], may be the result of procedural and/or species differences. The increases in punished behavior in the current study were not the result of any general stimulant activity of inosine. *d*-Amphetamine increased low rates of unpunished responding but only further decreased punished responding (data not shown) as reported previously for central nervous system stimulants [2, 5, 14].

The benzodiazepine-like behavioral effects of inosine in the present study may be a function of the interaction of inosine with benzodiazepine receptors in pigeon brain [17]. Ro 15-1788 reversed the inosine-induced increases in punished and unpunished responding at 0.03 mg/kg, a dose that had no effect when given alone. Although higher doses of Ro 15-1788, when given in combination with a rate-increasing dose of inosine, decreased responding much like Ro 15-1788 alone, such an interaction also occurs with benzodiazepines and Ro 15-1788 [25]. However, in contrast to the rate-increasing effects of inosine, combinations of Ro 15-1788 with a rate-decreasing dose of inosine revealed that a benzodiazepine receptor mechanism for the behavioral effects of high inosine doses does not exist; rate-decreasing effects of relatively high midazolam doses are completely reversed by Ro 15-1788 even at doses that decrease responding when given alone [25]. Evidence suggesting that inosine interacts with a barbiturate recognition site of the benzodiazepine-GABA receptor complex [10, 18, 19] is compatible with the observed increases in punished behavior by inosine but cannot account completely for the interaction of inosine and Ro 15-1788.

Inosine has been reported to behave like a benzodiazepine antagonist under a number of experimental conditions (e.g., [6,11]). However, in the current study punished responding was increased by inosine, an effect also found after benzodiazepine treatment but absent after administration of the benzodiazepine antagonists Ro 15-1788 or CGS 8216 [3, 23, 25]. Although Ro 15-1788 (0.01 mg/kg) increased unpunished responding as previously reported in pigeons [25], increases in punished responding were not obtained, and high doses decreased both punished and unpunished responding. While relatively low doses of Ro 15-1788 decrease responding of pigeons (see also [25]), rate-decreasing effects of inosine were obtained only after relatively large doses. Thus, behavioral effects of inosine do not resemble those of a benzodiazepine antagonist. A similar conclusion was reached in an experiment in which inosine, unlike Ro 15-1788, did not reverse or prevent the hypnotic effects of diazepam [26]. Thus, the increases in punished behavior observed here are consistent with the benzodiazepine agonist activity previously reported for inosine [12,21].

In summary, inosine produces effects on punished and unpunished behavior of pigeons which resemble those produced by benzodiazepines. However, these effects are unlike those of the benzodiazepine antagonists Ro 15-1788 or CGS 8216. The rate-increasing effects of inosine may be initiated by binding to benzodiazepine receptors while the rate-decreases produced by higher doses appear to be the result of non-benzodiazepine receptor mechanisms. Although doubts have been raised regarding the possibility that inosine functions as a physiological regulator of the benzodiazepine receptor (cf. [8]), the pharmacology of this nucleoside, with its dual ability to behave as an agonist and antagonist, reflect interesting aspects of benzodiazepine action and benzodiazepine receptor-coupled processes.

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