Imidazenil, a Positive Allosteric GABA<sub>A</sub> Receptor Modulator, Inhibits the Effects of Cocaine on Locomotor Activity and Extracellular Dopamine in the Nucleus Accumbens Shell Without Tolerance Liability<sup>1</sup>

MARCO GIORGETTI<sup>2,3</sup>, JAVAID I. JAVAID<sup>2</sup>, JOHN M. DAVIS<sup>2</sup>, ERMINIO COSTA<sup>2,3</sup>, ALESSANDRO GUIDOTTI<sup>2,3</sup>, SARAH B. APPEL<sup>4</sup> and MARK S. BRODIE<sup>4</sup>

The Psychiatric Institute, College of Medicine, University of Illinois at Chicago, Chicago, Illinois

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ABSTRACT

Imidazenil, a benzodiazepine recognition site ligand that acts as partial positive allosteric modulator of γ-aminobutyric acid (GABA) action at GABA<sub>A</sub> receptors, inhibits in a dose-dependent manner (0.56–2.5 μmol/kg i.p. to rats) the cocaine-induced increase in dopamine (DA) content in the dialysates of the nucleus accumbens shell and striatum and also inhibits cocaine-induced locomotor activity. Diazepam, a full allosteric modulator of GABA action at GABA<sub>A</sub> receptors, in a dose of 4.4 μmol/kg i.p. also attenuates the cocaine-induced increase in DA content in the dialysates of nucleus accumbens shell, and striatum and the cocaine-induced locomotor activity. However, imidazenil (2.5 μmol/kg i.p.) fails to reduce spontaneous locomotor activity, whereas diazepam (4.4 μmol/kg i.p.) elicits sedation and ataxia and clearly impairs spontaneous locomotor activity. When added in vitro, both imidazenil and diazepam potentiate the GABA-mediated reduction of the ventral tegmental area DA neuron firing rate. After protracted treatment (14 days/three times a day with an increasing-dose schedule), the inhibitory actions of imidazenil fail to develop tolerance, whereas the actions of diazepam exhibit high tolerance liability. We conclude that imidazenil is devoid of tolerance liability and that, via a GABA<sub>A</sub>-mediated reduction in the extracellular DA in nucleus accumbens shell, it might reduce the psychomotor activity and reinforcing properties of cocaine.

The psychomotor-stimulating and reinforcing properties of cocaine are believed to depend on the blockade of DA reuptake in brain areas innervated by DA neurons (Koob and Bloom, 1988; Wise, 1996).

In the rat striatum, the cocaine-mediated increase in extracellular DA and the consequent stimulation of postsynaptic DA receptors are primarily associated with the induction of stereotyped behavior (LeMoal and Simon, 1991), whereas in the ventral striatum (nucleus accumbens) the cocaine effect on DA is related primarily to the onset of both the increase of locomotor activity and the reinforcing properties (Maldonado et al., 1993; Ikemoto et al., 1997; Koob and Bloom, 1988; LeMoal and Simon, 1991).

These effects, which are due to the cocaine-induced blockade of the DA transporter (Kuhar et al., 1991), are highly dependent on the rate and amount of DA released from intraneuronal vesicular stores via impulse flow-dependent exocytosis (Florin et al., 1995; White, 1996). Thus, it is possible that the endogenous negative modulation of the firing rate of DAergic neurons may attenuate the rewarding and the reinforcing actions of cocaine. Although in vitro cocaine applied to SNc or VTA DAergic neurons inhibits their firing rate, systemic administration of behaviorally active doses of cocaine produce only a relatively weak inhibitory effect on VTA DA neurons, compared with the stronger enhancement of DA neurotransmission within the nucleus accumbens; the latter effect predominates, and this explains the extremely potent rewarding effects of cocaine (Einhorn et al., 1988). However, the firing rate of DAergic SNc-striatal and VTA-accumbens neurons is under potent trans-synaptic inhibitory control by GABA-releasing neurons that impinge on DAergic neuronal somata (Kalivas, 1993; Wise, 1996).

Interestingly, the BZD-mediated amplification of GABA

ABBREVIATIONS: DA, dopamine; DAergic, dopaminergic; GABA, γ-aminobutyric acid; VTA, ventral tegmental area; NAS, nucleus accumbens shell; ANOVA, analysis of variance; AUC, area under the curve; FAM, full allosteric modulator; PAM, partial positive allosteric modulator; aCSF, artificial cerebrospinal fluid; SAM, selective allosteric modulator; SNc, substantia nigra pars compacta; BZD, benzodiazepine.
action (Choi et al., 1981) at GABA_A receptors (Vicini et al., 1987) by FAMs BZDs (Giusti et al., 1993; Costa and Guidotti, 1996) also attenuates cocaine-induced psychomotor stimulant activity in humans and rats, reduces the cocaine-induced increase in DA content in striatal dialysates (Dewey et al., 1997) and reduces cocaine self-administration in rats (Goeders et al., 1993). These data suggest that FAMs be tested in the treatment of craving and other symptoms of cocaine abuse. However, protracted use of this class of drugs is considerably limited by the following untoward effects: 1) in rats they reduce cocaine-induced psychomotor activation and cocaine self-administration in doses that are close to those eliciting sedation and ataxia, and 2) their repeated administration produces tolerance, which eliminates their beneficial effects.

We hypothesized that to reduce cocaine-induced psychomotor activation and cocaine-elicited DA increase in striatal and NAS microdialysates, one could use imidazenil, a potent anxiolytic imidazo-benzodiazepine endowed with partial positive allosteric modulatory activity of GABA action at many GABA_A receptor subtypes, which is virtually devoid of tolerance and dependency liability (Auta et al., 1994; Ghiani et al., 1994; Impagnatiello et al., 1996; Costa and Guidotti, 1996).

The aim of the present study was to compare imidazenil (a PAM) and diazepam (a FAM) in terms of their potency to 1) inhibit the cocaine-induced increase in DA content in the NAS and in striatal dialysates, 2) inhibit the cocaine-induced increase in locomotor activity, 3) potentiate the GABA-elicited inhibition of VTA DAergic neurons firing rates in vitro and 4) elicit tolerance liability to the antagonism of cocaine.

We report here that single doses of either imidazenil or diazepam attenuate the cocaine-induced increase in locomotor activity and in DA content in the dialysate of the NAS and striatum of freely moving rats and that they potentiate GABA-induced inhibition of the firing rate of VTA DA neurons in vitro. Moreover, whereas tolerance to these effects followed protracted treatment with diazepam, protracted treatment with imidazenil (see “Materials and Methods”) failed to produce tolerance.

Materials and Methods

Animal housing and surgery. Male Fischer 344 rats (Harlan) were housed individually with a 12/12-hr light/dark cycle; food and water were available ad libitum. Cocaine injections (Sigma Chemical Co., St. Louis MO), behavioral testing and dialysis experiments were performed between 9 A.M. and noon.

Before surgery, rats weighing 230 to 270 g were anesthetized with sodium pentobarbital (200 μmol/kg) (Abbott Laboratories, IL); dialysis probes were then implanted at the following coordinates relative to bregma and dura: AP + 0.7 mm, L −3.0 mm, D/V −5.5 mm (for the right striatum); or in the right NAS: AP +2.0 mm, L −1.0 mm, D/V −7 mm. These coordinates were calculated according to the Paxinos and Watson atlas for the rat brain (Paxinos and Watson, 1986).

Dialysis probes were constructed using silica capillary tubing, and the dialysis fiber (I.D. 0.22 mm, O.D. 0.31 mm, 4 mm long) was prepared from polyacrylonitrile/sodium methyal sulfate copolymer with a cutoff of 13,000 DA (Blandina et al., 1996).

All experimental procedures were carried out in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Care and Use of Laboratory Animals Committee.

Microdialysis experiments. Twenty-four hours after probe implantation, rats were connected to a microperfusion pump (Harvard apparatus) with a microdialysis syringe (CMA model, 1-ml vol), and the striatum or the NAS was perfused using artificial cerebrospinal fluid (aCSF) composed of NaCl 145 mM, KCl 2.7 mM, MgCl₂ 1 mM, CaCl₂ 1.2 mM, and Na₂HPO₄ 2 mM, pH 7.4.

The perfusion flow rate was set at 2 μl/min with a collection time for each sample of 20 min, such that 40 μl of perfusate was collected and analyzed using an HPLC coupled to an electrochemical detector.

Measurement of DA levels in microdialysis samples. The perfusate was assayed for DA content using an HPLC coupled to an ESA coulochem II 5200 A detection system with oxidation potential +320 mV at range of 50 nA. The detector was equipped with a high-performance analytical cell (ESA model 5014) tailored for use in the analysis of dialysates. The mobile phase was composed of 0.05 M monobasic sodium phosphate, 0.1 N sodium acetate and 1% methanol and was adjusted to pH = 4.4 with HPLC grade phosphoric acid. The flow rate of the system was 1.0 ml/min. The limit of detection for DA was approximately 3 fmol.

DA levels in each sample were expressed as the percentage of baseline release measured as the mean of the first 3 to 5 samples collected immediately before treatment with drugs.

Histological analysis. At the completion of microdialysis experiments, the animals were given an overdose of pentobarbital and perfused through the heart with phosphate-buffered saline followed by 10% formalin/isotonic saline. Brain coronal slices (100 μm) were stained with cresyl violet, and probe placement was determined by light microscopy in the dorsal striatum (not shown) and in the NAS. As an example, in figure 1, we have diagrammatically represented

Fig. 1. Location of the tips of the microdialysis probes in the NAS. Data relative to the experiment described in figure 2. The numbers indicate millimeters rostral to the bregma according to the Atlas of Paxinos and Watson (1986). The microdialysis probes in the shell were localized (●) to the dorso-medial portion but not to the ventral portion. The figure also indicates the incorrect placements (●) relative to the animals that were discarded.
all probe placements in NAS relative to the experiment described in figure 2. These placements were similar in all the reported experiments. Animals in which the probe was located outside the target area were discarded.

**Locomotor activity.** Spontaneous and cocaine-induced locomotor activity in rats was measured using the Photobeam Activity System (San Diego Instruments, Inc.) linked to an IBM 386 computer. A clear Perspex cage (42 cm × 23 cm × 17 cm) containing a small amount of sawdust was surrounded by a metal frame with seven infrared beams and sensors placed 2 in. apart. Total activity (total number of beam breaks) was selected as the measure of locomotion. Visual observation by the operator confirmed that locomotion and not stereotypy was the cause of the behavior. Before the test period, the animals were kept three to a cage in their home cages. Rats were allowed a 15-min habitation period in the activity cages and then were treated intraperitoneally i.p. with vehicle, diazepam or imidazeni. After a 40-min recording period, some rats received an i.p. injection of cocaine, and the locomotor activity was measured for another 40-min period. Locomotor activity was measured between 9 A.M. and noon.

**Schedule for long-term diazepam and imidazeni treatment.** Diazepam and imidazeni (Hoffmann-La Roche, Nutley, NJ) were suspended in water containing 0.05% Tween 20 (Sigma Chemical Co., St. Louis, MO) and administered in 1-ml volume by oral gavage three times a day (approximately at 9.00 A.M., 2.00 P.M., and noon). Imidazeni and diazepam were prepared as previously described (Brodie et al., 1994; Impagnatiello et al., 1996). Control rats received only vehicle. In rats receiving vehicle, diazepam or imidazeni long-term treatment, the microdialysis probe was implanted at the 14th day of treatment, and treatment was discontinued at day 15.

**Extracellular recording of VTA neuron firing rate in brain slices.** Brain slices from rats (Fischer 344, 100–200 g) containing the VTA were prepared as previously described (Brodie et al., 1990, 1995; Mueller and Brodie, 1989). Rats received long-term diazepam or imidazeni treatment with the schedule described above.

Coronal sections (400 μm thick) were cut on a Lancer vibratome, and the tissue was placed directly in the recording chamber. Small platinum weights were placed on the slice to increase the stability of the recordings. The slice was covered with medium, and a superfusion system maintained the flow of medium at 2 ml/min; the temperature in the recording chamber was kept constant at about 35°C. The composition of the aCSF in these experiments was as follows (in mM): NaCl 126, KCl 2.5, NaH2PO4 1.24, MgSO4 1.3, CaCl2 2.4, NaHCO3 26, glucose 11; the aCSF was saturated with 95% O2/5% CO2 (pH = 7.4). The flow rate was continuously monitored with a flowmeter, and adjustable valves were used to keep the rate constant. The small volume chamber (about 300 μl) used in this study permitted the rapid infusion and washout of drug solutions.

Drugs were added to the aCSF in the fluid delivery tubing by means of a calibrated infusion pump from stock solutions 100 to 1000 times the desired final concentrations. Final concentrations were calculated from aCSF flow rate, pump infusion rate and concentration of drug stock solution. Infusion of drug solutions never exceeded 1% of the flow rate of aCSF. GABA was dissolved in degassed distilled water; diazepam and imidazeni were dissolved in the same vehicle used for chronic treatment.

Extracellular recording electrodes were made from glass tubing 1.5 mm in diameter; the tip resistance of the microelectrodes ranged from 4 to 8 MΩ. At least 1 hr was allowed for equilibration after preparation of the slice. After this period, the electrode was lowered into the VTA under visual guidance. The VTA is clearly visible in fresh tissue as a gray area medial to the substantia nigra. All neurons included in this study conformed to criteria for putative DAergic neurons established in this laboratory (Brodie et al., 1990) (Brodie and Dunwiddie, 1987) and others (Lacey et al., 1989) (Grace and Bunney, 1980) (Grace and Bunney, 1983). These criteria included a slow, regular firing rate (0.5–5 Hz) and a long-duration (>2.5 msec) action potential, often with an inflection on the rising phase.

Frequency of firing was determined with a window discriminator and ratemeter, the output of which was fed to a chart recorder. In addition, an IBM-PC-based data acquisition system was used to calculate, display and store the frequency of firing over 5-sec and 1-min intervals. Each neuron served as its own control; drug responses were quantitated as the mean change in firing rate (normalized as a percentage of control) over a 1-min interval during the peak of the drug response. The calculation to normalize the GABA-elicited firing rate (FR) decrease is

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\text{GABA FR} = \frac{\text{Basal FR} - \text{Drug FR}}{\text{Basal FR}} 
\]

![Fig. 2. A) Imidazeni (Imid) attenuates the cocaine-induced increase in DA in the NAS in a dose-related manner. This inhibition by imidazeni of the cocaine-elicited increase in DA in NAS dialsates is significant at doses of 0.56 ( – ), 1.2 (– – ) and 2.5 μmol/kg i.p. ( – – ). Dunnett’s post-hoc test: P = .02, .005 and .001 respectively. This test evaluates each dose vs. the vehicle (Veh)-treated group ( – ). Each point represents the mean ± S.E.M. of 3 to 5 animals. B) Diazepam (Diaz) (4.4 μmol/kg i.p.) (– – ) significantly attenuates cocaine-induced increase in DA content in NAS dialsates when compared with the vehicle (Veh)-treated group ( – )]. t test; P = .02. Statistical analyses were performed by comparing the different AUC. In the experiments of figure 2A and B, basal levels of DA averaged 55 ± 14 fmol/sample.](image-url)
This procedure, which we have used in the past (Brodie et al., 1990) and which has proved reliable, is intended to control for minor spontaneous shifts in FR. For each VTA neuron, we computed the amplitude of GABA inhibition in the presence or absence of diazepam or imidazenil.

Data analysis. In the microdialysis experiments, DA recovery averaged 9% to 12% through the microdialysis probe. These data agree with those reported by others (Benveniste and Huttemeier, 1990). Measured levels were expressed as a percentage of the basal release (an average of 3–5 samples collected before drug administration).

Linear regression constructed from appropriate standards was used to estimate the perfusate amounts of DA released during the 20-min collection period. Microdialysis and locomotor activity data were analyzed by taking the AUC obtained for each dose of tested drug. We then applied a general linear model for ANOVA to determine the differences between the curves. Each dose level was compared to vehicle control using Dunnett's test for post-hoc analysis. We predicted that tolerance to diazepam would occur but not tolerance to imidazenil. Therefore, animals chronically treated with diazepam and then challenged with diazepam would be indistinguishable from controls, but animals chronically treated and challenged with imidazenil would have decreased DA levels. Hence we evaluated this experiment using an ANOVA with Helmert contrasts, a specific post-hoc test of whether the imidazenil-treated animals had decreased DA levels below those of the other groups and whether the other two groups had equal DA levels. When only two groups were compared, the t test for independent groups was used. All mean values are expressed as the mean ± S.E.M. Dose response was tested by linear regression of dose vs. response.

The electrophysiological data were analyzed with ANOVA to determine differences between GABA responses in the presence of vehicle or BZDs (diazepam or imidazenil). The Newman-Keuls test was used for post-hoc analysis (SigmaStat, Jandel Scientific, San Rafael, CA). All values are expressed as mean ± S.E.M.

Drugs. Imidazenil and diazepam (Hoffmann-LaRoche, Nutley, NJ) solutions were freshly prepared before each microdialysis, locomotor and electrophysiological activity experiment. The drugs were dissolved in a medium of propylene glycol 50% (Fisher Sci, Itasca, IL), polyethylene glycol 11% (Sigma Chemical Co., St. Louis, MO), H2O 37% and dimethyl sulfoxide, 2% (Sigma). Cocaine hydrochloride (Sigma, St. Louis, MO) was dissolved in isotonic saline for systemic injection. For the acute studies, imidazenil, diazepam and cocaine were always administered i.p.

Results

Both imidazenil and diazepam attenuate the cocaine-induced increase in the DA content of NAS dialysates. The i.p. administration of 45 μmol/kg of cocaine resulted in a 3-fold increase in DA concentration in the dialysates from the NAS. This effect peaked at about 20 min and then began to decline, reaching basal levels in about 60 min (fig. 2). Imidazenil attenuated the cocaine-induced DA increase in a dose-dependent manner (f = 30.1; dF = 1, 13; P < .001; fig. 2A) (F test for the linear portion of the dose-response curve). More specifically, the overall increase in the dialysate DA content produced by cocaine in vehicle-treated rats was decreased by approximately 14%, 33%, 42% and 56% (values were calculated by considering the various AUC) by 0.31, 0.56, 1.2 and 2.5 μmol/kg of imidazenil, respectively (fig. 2A). Imidazenil's inhibition of the cocaine-elicited DA increase in NAS dialysates is significant at doses of 0.56, 1.2 and 2.5 μmol/kg (Dunnett's post-hoc test; P = .02, .005 and .001, respectively). Like to imidazenil, diazepam administered i.p. 40 min before cocaine injection (45 μmol/kg) attenuated the cocaine-induced DA increase in the dialysates of the NAS (fig. 2B). More specifically, a single dose of diazepam (4.4 μmol/kg) reduced the cocaine-induced DA increase in NAS dialysates by about 41% (t = 2.8; dF = 12; P = .02; fig. 2B).

Both imidazenil and diazepam attenuate the cocaine-induced increase in the DA content of striatal dialysates. The i.p. administration of 45 μmol/kg of cocaine resulted in a 2-fold increase in striatal dialysate DA content. This effect reached a peak between 20 and 40 min and then declined toward basal level in about 60 min (fig. 3). Imidazenil administered i.p. to rats 40 min before cocaine injection in a dose (2.5 μmol/kg) that itself failed to alter the DA striatal dialysate content (fig. 3A) reduced the AUC of the cocaine-induced DA increase in the striatal dialysates by almost 50% (t = 3.2; dF = 12; P < .01; fig. 3A). Like imidazenil, a single dose of diazepam (4.4 μmol/kg) administered i.p. to rats 40 min before cocaine injection reduced the cocaine-induced DA increase in the dialysates by almost 50%. The difference in AUC was significant (t = 2.8, dF = 12; P < .02 fig. 3B).

Inhibition by imidazenil of the cocaine-induced increase in DA content fails to show tolerance. When rats receiving long-term treatment with increasing doses of diazepam or pharmacologically equieffective doses of imidazenil or vehicle (see "Materials and Methods" for drug schedule) were left drug-free for 18 hr after the last injection, they failed to show differences in basal or cocaine-induced in-

![Image](https://jpet.aspetjournals.org/doi/fig/10.1093/jpet/21.1.61)
crease in the DA content of NAS or striatal dialysates (data not shown). This result confirms previously reported findings that diazepam and imidazenido were completely eliminated from the rat brain 18 hr after the last injection (Impagnatiello et al., 1996). At this time, a challenge with imidazenido (2.5 μmol/kg) was fully effective in attenuating the cocaine-induced increase in DA content observed both in the NAS (fig. 4A) and in the striatal dialysates (fig. 5A) of freely moving rats receiving long-term treatment with either vehicle or with imidazenido. In contrast, a similar challenge with diazepam (4.4 μmol/kg) failed to be effective in reducing the cocaine-induced increase in DA content in either NAS (fig. 4B) or striatal dialysates (fig. 5B).

Attenuation by imidazenido of cocaine-induced locomotor activity fails to show tolerance. In separate groups of animals without implantation of the microdialysis probe, we also studied the effects of diazepam and imidazenido on spontaneous and cocaine-induced locomotor activity.

Figure 6, A and B, shows that cocaine (45 μmol/kg) administered 18 hr after 14 days of repeated treatment with diazepam or imidazenido produced an increase in locomotor activity similar to the increase observed in vehicle-treated animals. Diazepam (4.4 μmol/kg, a dose that attenuates cocaine-induced increase in DA content of NAS dialysates) reduced by approximately 50% the spontaneous locomotor activity and virtually abolished cocaine-induced locomotor activity (P < .01) in long-term vehicle-treated rats, but the same dose had only marginal effects on spontaneous and cocaine-induced locomotor activities in rats treated for 14 days on a dose schedule of diazepam (P = .08; fig. 6B) (see “Materials and Methods”). Conversely, the inhibitory efficacy of imidazenido (2.5 μmol/kg) on cocaine remained unabated in both vehicle-treated rats and rats receiving the 14-day imidazenido schedule (P < .001 in both cases; fig. 6A). This dose of imidazenido failed to induce change in spontaneous locomotor activity in long-term vehicle-treated or imidazenido-treated rats.

Potentiation by imidazenido of GABA inhibition of VTA DAergic neuron firing is devoid of tolerance. After long-term diazepam or imidazenido treatment (see “Materials and Methods”), the ability of either of these BDZ recognition site ligands to potentiate the GABA inhibition of VTA DAergic neuron firing was tested electrophysiologically. Extracellular single-unit recordings were obtained from DAergic VTA neurons in brain slices prepared from rats treated long-term with diazepam or imidazenido according to the protocol in “Materials and Methods.” Figure 7 illustrates that in control rats, the reduction in firing rate elicited by 10 μM GABA is greatly potentiated by an addition of 5 μM diazepam. In contrast, as shown in the upper part of figure 8, after long-term treatment with diazepam, the GABA-elicted reduction in DA neuronal firing rate is only marginally increased in the VTA.

The graph in the lower part of figure 8 illustrates pooled data from rats receiving long-term treatment with diazepam and shows that the extent of GABA inhibition fails to change significantly in the presence of either 1 μM diazepam (■) or 5 μM diazepam (●) (two-way ANOVA; P > .05). In this graph, the response (percent inhibition) of each cell to GABA concentration (10–100 μM) in the absence of diazepam was subtracted from the response of the same neuron to the application of GABA in the presence of 1 μM or 5 μM diaz-
epam. The mean differences are plotted here as a function of the GABA concentration. Therefore, zero would represent no change in GABA potency, and the negative values indicate the degree of GABA potentiation elicited by diazepam.

Similar tests were carried out after long-term treatment with imidazenil. The upper part of figure 9 illustrates the potentiation of 10 μM GABA on a VTA neuron from a rat receiving long-term treatment with imidazenil.

The lower part of figure 9 illustrates pooled data from VTA preparations from rats receiving 14 days of treatment with imidazenil and shows that both 1 μM (●) and 5 μM (■) doses of imidazenil enhance GABA inhibition (two-way ANOVA, P < .001, n = 5) (fig. 9). The potentiation of GABA inhibition induced by imidazenil is comparable to that obtained by diazepam in control rats (compare figs. 7 and 9). Thus these studies demonstrate that imidazenil is devoid of tolerance liability but that tolerance to diazepam develops after protracted diazepam treatment.
Discussion

The following structural requirements define the susceptibility of GABA_A receptor subtypes to the allosterically elicited amplification of GABA action by anxiolytic BZD: 1) the presence of at least one among \( \alpha_1, \alpha_2, \alpha_3 \) and \( \alpha_6 \) GABA_A receptor subunits; 2) the presence of the S or L variants of \( \gamma_2 \) subunits; 3) the presence of one or more \( \beta \) subunits and 4) the absence of the \( \alpha_6 \) receptor subunit (Puia et al., 1991; Dötsch et al., 1995; MacDonald and Olsen, 1994; Sieghart, 1995). In the framework of these GABA_A receptor structural requirements, the chemical structure of anxiolytic BZDs confers particular profiles of their pharmacological action. In the introduction, we defined the different activity profiles of PAM and FAM anxiolytic BZD (Giusti et al., 1993; Costa and Guidotti, 1996). It is important to differentiate PAM BZD from another class of BZDs that maximize the amplification of GABA action in selective GABA_A receptor subtypes, including a specific \( \alpha \) subunit. An example of this class of SAM is zolpidem, which by selectively amplifying GABA action in receptors, including the \( \alpha_1 \) subunit, facilitates the onset of sleep. Such a differentiation is important, because unlike PAMs, the pharmacological action of SAMs includes tolerance and dependence liability when they are given in high doses. In contrast, PAMs, even when given in doses that are multiples of those that are maximally active, fail to elicit tolerance and dependence liability (Costa and Guidotti, 1996).

From the standpoint of the therapeutic use of BZDs, the lack of tolerance and dependence liability confers a unique characteristic on PAMs (Impagnatiello et al., 1996; Costa and Guidotti, 1996). Despite claims to the contrary, there are only a few known BZDs that, on the basis of experimentation in rats and monkeys, can be considered PAM BZDs devoid of tolerance and dependence liability (Costa and Guidotti, 1996). One of them is imidazenil, a PAM that fails to be recognized FAMs, imidazenil in doses 250 times greater than the pharmacologically active doses fails to generalize to classic BZDs with FAM activity (Paronis et al., 1997).

In the present experiments with freely moving rats, we found that the effect of a single imidazenil dose on the cocaine-elicited increase in DA content in NAS dialysate was to inhibit the action of cocaine in a dose-dependent manner (fig. 2A). Similarly, the cocaine-elicited increase in DA content in NAS dialysates can be inhibited by appropriate doses of diazepam (fig. 2B). Also a single dose of either drug can...
drug that also acts at GABA\textsubscript{A} receptors but fails to produce spontaneous motor activity. However, we show that imidazenil, a specific or simply reflects the consequences of reduced synthesis that imidazenil may increase the efficacy of the smaller substance to the development of tolerance; in contrast, the efficacy of GABA inhibition is potentiated by imidazenil in long-term imidazenil-treated rats. VTA neurons in slices from five rats were tested with various concentrations of GABA in the presence of vehicle and two concentrations of imidazenil. The spontaneous firing rate of VTA neurons was decreased by GABA in a concentration-dependent manner. In the top portion of the figure are firing-rate histograms recorded from the same VTA neuron in vehicle (top left) or in 5 \textmu M imidazenil (top right). The horizontal bar represents the duration of application of GABA (10 \textmu M). In vehicle, 10 \textmu M GABA produced a 13% decrease in firing rate, and in imidazenil, 10 \textmu M GABA produced a 63% decrease in firing rate. The bottom portion of this figure illustrates the response of the population of cells tested. To present the mean data concisely, we subtracted the response (percent inhibition) to GABA in the absence of imidazenil from the GABA response of the same neuron in the presence of imidazenil. The means of these differences are plotted as a function of GABA concentration for cells from rats treated chronically with imidazenil. A value of zero means of these differences are plotted as a function of GABA concentration for cells tested. To present the mean data concisely, we subtracted the response (percent inhibition) to GABA in the absence of imidazenil from the GABA response of the same neuron in the presence of imidazenil. The means of these differences are plotted as a function of GABA concentration for cells from rats treated chronically with imidazenil. A value of zero represents no change in GABA potency produced by imidazenil. The potency of GABA was significantly increased by imidazenil (two-way ANOVA, \( P < .001, n = 5 \)). Both concentrations of imidazenil (1 \textmu M and 5 \textmu M) significantly increased the potency of GABA (Student-Newman-Keuls, \( P < .05 \)).

Thus, because imidazenil continues to inhibit efficaciously the increased motor activity elicited by cocaine, the hypothesis that imidazenil may increase the efficacy of the smaller amounts of DA that are increased in the NAS and striatum...
dialysates after cocaine treatment does not appear to be tenable.

In conclusion, the present experiments suggest that treatment with imidazenil might be tested in the self-administration of cocaine in rats and monkeys to evaluate its ability to reduce cocaine craving in patients dependent on cocaine. We have initiated these studies on the action of imidazenil in the self-administration of cocaine in rats. So far, imidazenil appears to reduce cocaine self-administration in rats without evidence of tolerance liability.

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Send reprint requests to: Erminio Costa, M.D., The Psychiatric Institute, 1601 W. Taylor St., Room 314 W, Chicago, IL 60612.