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Lack of anticonvulsant tolerance and benzodiazepine receptor down regulation with imidazenil in rats

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1 Development of anticonvulsant tolerance and benzodiazepine (BZD) receptor down-regulation has been reported to occur upon chronic administration of conventional BZDs. We compared the effect of chronic treatment with imidazenil, a new BZD partial agonist, and diazepam in rats.

2 After acute administration, imidazenil was more potent though less effective than diazepam in protecting from bicuculline-induced seizure. The time-course analysis of two peak equieffective doses of imidazenil (2.5 μmol kg $^{-1}$ p.o.) and diazepam (35 μmol kg^{-1}, p.o.) showed a longer lasting action of the former drug.

3 The anticonvulsant efficacy of diazepam (35 μmol kg^{-1}, p.o.) was reduced in rats given chronic diazepam (35 μmol kg^{-1} p.o., 3 times a day for 8-15 days). No tolerance to imidazenil (2.5 μmol kg^{-1}, p.o.) was apparent after 130-day administration with imidazenil (2.5 μmol kg^{-1}, p.o., 3 times a day).

4 Plasma levels of imidazenil and diazepam, assessed 30 min after administration, were not changed in chronically treated animals.

5 In rats made tolerant to diazepam, the maximum number of [^{3}H]-flumazenil binding sites were reduced in both cerebral cortex (-36%) and cerebellum (-42%). No changes in [^{3}H]-flumazenil binding were found in chronic imidazenil-treated rats.

6 Specific [^{3}H]-flumazenil binding *in vivo* was decreased in the forebrain of chronic diazepam- but not of chronic imidazenil-treated animals.

7 These data indicate that imidazenil possesses a very low tolerance potential to its anticonvulsant activity and does not affect BZD receptor density even after prolonged administration.

Keywords: Imidazenil; diazepam; benzodiazepine; partial agonist; anticonvulsant; chronic treatment; tolerance

Introduction

Though benzodiazepines (BZDs) possess excellent anticonvulsant activity and tolerability, their usefulness is limited in maintenance therapy of epilepsy because tolerance develops to their anticonvulsant effects following chronic treatment (Robertson, 1986; Schmidt *et al.*, 1986; Haigh & Feely, 1988a). It has been recently suggested that partial agonists at the BZD receptor may have advantages over the more conventional full agonists regarding both their spectrum of action and their relative tolerance/dependence-inducing potential (Haefely *et al.*, 1990). In support of this it has been found that a number of partial agonists exhibited no significant tolerance in animal models in which conventional BZDs show varying degrees of tolerance when tested over a similar period (Boast & Gerhardt, 1987; Haigh & Feely, 1988b; Feely *et al.*, 1989). Imidazenil, an imidazobenzodiazepine carboxamide derivative, has been shown to be a BZD receptor ligand exhibiting potent anticonvulsant and anxiolytic activity in rodents (Giusti *et al.*, 1993; Serra *et al.*, 1994a). Evidence from a number of *in vitro* and *in vivo* tests indicated that this compound belongs to the class of partial agonists (Guisti *et al.*, 1993; Serra *et al.*, 1994a). For instance, when tested on a broad spectrum of recombinant $GABA_A$ receptor subtypes, imidazenil acts with lower efficacy than diazepam on GABA-gated Cl^- currents and a large fractional receptor occupancy *in vivo* is required for imidazenil to produce the same anxiolytic activity of the full agonists diazepam and alprazolam. Auta *et al.* (1994) found that a 2-week treatment with diazepam and triazolam, but not imidazenil, rapidly induced tolerance to the protective effect of these drugs against bicuculline-induced convulsions. No tolerance to the ability of imidazenil to antagonize the convulsions and the increase in [^{35}S]-TBPS binding to $GABA_A$ receptor elicited by isoniazid in mice has been reported after one month administration (Ghiani *et al.*, 1994). It was however hypothesized that induction of tolerance to the effects of a partial agonist like imidazenil may require a much longer interval of treatment than for a full agonist (Miller *et al.*, 1990).

In the present study we were interested in examining whether anticonvulsant tolerance develops after a prolonged (several weeks) treatment with imidazenil. As the acute actions of BZDs are thought to be mediated by GABA (Tallman & Gallager, 1985), efforts to elucidate the neuronal adaptations underlying behavioral tolerance have focused on changes in GABAergic processes. Several mechanisms that have been proposed for BZD tolerance are based on findings from receptor binding studies which indicated changes occurring at various sites on the GABA/BZD/Cl^- channel complex. These findings include down-regulation of BZD binding sites (Grimm & Hershovitz, 1981; Rosenburg & Chiu, 1981; Tietz *et al.*, 1986; Miller *et al.*, 1988). Therefore we also evaluated changes in BZD receptor binding after chronic imidazenil, both *in vitro* and *in vivo*. A conventional full agonist BZD, diazepam, was included in the study, for the purpose of comparison.

Methods

Animals

Male Sprague-Dawley rats (Charles River, Italy) weighing 150-175 g at the beginning of the experiment were housed in groups of five animals and maintained on a 12 h light-dark cycle with free access to standard diet and water.

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Convulsive test procedure

Bicuculline (0.24 μmol ml^{-1}) was given by infusion (0.42 ml min^{-1}) into a tail vein of the unrestrained rat until a tonic-clonic convulsion was elicited; this convulsion represented the end-point of the test and rats were killed immediately after it had occurred. The minimum convulsant dose of bicuculline required to elicit this seizure was obtained for each rat and the mean (± s.e. mean) calculated for each group. In rats that failed to convulse because of treatment with imidazenil and diazepam the infusion was stopped after 15 min.

Acute anticonvulsant study

Dose-response curves for acute anticonvulsant activity of imidazenil and diazepam were obtained by giving either of the compounds 30 min before bicuculline infusion. A time-course analysis was conducted by giving the minimum dose of imidazenil producing maximal protection from seizures and a similar effective dose of diazepam at 30, 60, 90, 120, 180, 240 and 300 min before the bicuculline infusion: the area under the curve (AUC) of the anticonvulsant activity was measured with a trapezoidal rule (Ritschel, 1986).

Anticonvulsant tolerance study

The minimum dose of imidazenil producing maximum protection from bicuculline-induced seizures at 30 min (2.5 μmol kg^{-1}) and an equivalent peak anticonvulsant dose of diazepam (35 μmol kg^{-1}) were chosen for evaluating tolerance. Drugs were administered 3 times daily (07 h 00 min, 13 h 00 min and 19 h, 00 min). Chronic treatment with each of the two compounds was terminated when tolerance was apparent, or after 130 days. From each treatment cohort, groups of five rats were taken at random and given bicuculline (convulsive test) at 1, 4, 8, 15, 30, 60 and 130 days. Rats were tested 18–20 h after the last drug administration since a preliminary study revealed a short-lasting (no longer than 48 h) tolerance to diazepam in our experimental conditions. On test days the anticonvulsant drugs were administered 30 min before bicuculline infusion. At the beginning of the study, additional groups of rats ($n=5$) were tested acutely with each drug and vehicle. On the final day of each drug treatment, groups of control rats ($n=5$) that had received vehicle treatment (5 ml kg^{-1}) on the same regimen as the imidazenil and diazepam-treated groups were given an acute dose of imidazenil, diazepam and vehicle and tested with bicuculline.

Determination of plasma concentrations

Eighteen hours after the last drug administration, an oral dose of imidazenil (2.5 μmol kg^{-1}) or diazepam (35 μmol kg^{-1}) was given to groups of 4 rats, chronically treated with imidazenil (2.5 μmol kg^{-1}, 3 times a day for 130 days) or diazepam (35 μmol kg^{-1}, 3 times a day for 15 days) respectively. Animals treated with vehicle for the same periods were used as controls. Imidazenil was extracted from plasma samples after a partition with diethyl ether using alprazolam as internal standard. The organic phase was evaporated to dryness and the residue was dissolved in mobile phase and injected into the high performance liquid chromatograph (h.p.l.c.) (Perkin-Elmer, Norwalk, CT, U.S.A.). The analyses were performed in normal-phase mode using a Lichrosorb-CN column (Merck, Darmstadt, D) and a mobile phase consisting of *n*-exane:ethanol:acetic acid (78:17.6:4.4 v/v/v) at a flow rate of 1.5 ml min^{-1}. The detector was operated at 255 nm.

Diazepam was extracted from plasma samples, after the addition of flunitrazepam as internal standard, using a solid phase extraction procedure. The samples were applied to a C18 cartridge (Sep-Pak, Waters, Milford, MA, U.S.A.), purified from impurities, and then methanol was used to elute diazepam and the internal standard. An aliquot of the extract was injected into the h.p.l.c. A μBondapak Phenyl column (Waters, Milford, MA, U.S.A.) was used, with a mobile phase composed of phosphate buffer 10 mM:acetonitril (62:38v/v) at a flow rate of 1.5 ml min^{-1}, with uv detection at 255 nm.

In vitro *binding experiments*

BZD binding was studied using [^{3}H]-flumazenil. Rats (3 animals per group) were killed 18 h after the last drug treatment. Different brain areas were rapidly dissected on ice and homogenized in 20 ml of ice-cold 0.32 M sucrose pH 7.4 in a glass homogenizer with a Teflon pestle (10 up and down strokes). The homogenate was centrifuged at 1,000 *g* at 4°C for 10 min, the P_1 pellet was discarded, and the supernatant was collected and recentrifuged at 20,000 *g* at 4°C for 20 min. The resulting crude mitochondrial pellet (P_2) was resuspended in 20 ml of ice-cold distilled water and homogenized. The homogenate was centrifuged at 8,000 *g* at 4°C for 20 min, the supernatant was collected and recentrifuged at 48,000 *g* at 4°C for 20 min, and the final crude microsomal pellet (P_3) was frozen for at least 24 h. The pellet was resuspended in 10 ml of 50 mM Tris-HCl pH 7.4, centrifuged at 48,000 *g* at 4°C for 20 min and then resuspended in 10 volumes of Tris-HCl pH 7.4 + NaCl mM + KCl 5 mM (incubation buffer) for standard binding assay. Aliquots of membrane suspension (100 μl, or 0.15 mg of protein) were added to incubation medium containing different concentrations of [^{3}H]-flumazenil (specific activity 72.4 Ci $mmol^{-1}$) in a final volume of 1 ml. The reactions were carried out at 0°C for 60 min. Incubations were carried out in triplicate and non specific binding measured in the presence of 1 mM clonazepam. Incubation ended by the addition of 5 ml ice-cold Tris-HCl followed by rapid filtration through Whatman GF/C glass fibre filters (Whatman Inc., Clifton, NJ, U.S.A.) and two additional washes. The radioactivity trapped on the filters was counted after the addition of 8 ml of Filter Count (Packard), by liquid scintillation spectrometry. Saturation binding isotherm were analysed either by Scatchard analysis (Scatchard, 1949) or a non linear regression computer programme (Sacchi-Landriani *et al.*, 1983).

In vivo *binding experiments*

The method used in this study was described by Giusti *et al.* (1993). Briefly, on the day of binding assay, 18 h after the last drug administration, chronic vehicle- and BZD-treated rats were split in groups of 3 animals each. Chronic vehicle-treated rats received acute i.v. injection of various doses of diazepam or imidazenil. Rats treated chronically with diazepam and imidazenil received various i.v. doses of diazepam and imidazenil, respectively. Seven min later all the rats were injected with [^{3}H]-flumazenil (50 μCi kg^{-1} i.v., 1 ml kg^{-1}). After 3 min animals were killed and forebrain rapidly excised. The tissues were homogenized in Tris-HCl buffer 50 mM, pH 7.4 (1/10 w/v). One ml of the homogenates was filtered immediately through Whatman filters and the filters were washed 3 times with 3 ml of cold buffer. Non-specific binding was defined as radioactivity bound to membrane when the rats were given 20 μmol kg^{-1} i.v. of clonazepam 10 min before they were killed. IC_{50} ± s.e. mean value for each experiment were calculated by a computer-assisted curve fitting programme (EBDA) (McPherson, 1987).

The fractional BZD binding site occupancy was calculated as follows:

Fractional occupancy (%) = $100/[1+(b/x)^c]$

Where $b=ID_{50}$ dose inhibiting specific [^{3}H]-flumazenil binding by 50% (μmol kg^{-1}); x = dose (μmol kg^{-1}) and c = slope factor.

Drugs and solutions

Imidazenil was synthesized by Hoffmann-La Roche Inc. (Nutley, NJ, U.S.A.). Diazepam was purchased from F.I.S. (Vicenza, Italy), alprazolam was a gift of Upjohn (Kalamazoo,

MI, U.S.A.) and flunitrazepam was obtained from Hoffmann-La Roche Inc. (Basel, Switzerland). [^{3}H]-flumazenil was obtained from NEN (Boston, MA, U.S.A.). Bicuculline was purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.). Bicuculline was dissolved in isotonic saline; imidazenil and diazepam were suspended in 0.05% Tween 80 (v/v in isotonic saline) by ultrasound dispersion. Imidazenil and diazepam were administered by oral gavage in a volume of 5 ml kg^{-1}.

Statistics

Results are expressed as means ± s.e. mean. Statistical analysis of anticonvulsant activity data was performed by analysis of variance (ANOVA); when statistical significance was obtained with ANOVA, a *post hoc* Dunnett's test was performed for multiple comparisons. In binding studies, significant differences between means were determined by Student's *t* test. The probability level chosen was $P < 0.05$.

Results

Effect of acute imidazenil and diazepam on bicuculline-induced convulsions

The anticonvulsant efficacy of a single dose of imidazenil and diazepam against bicuculline-induced seizures is shown in Figure 1. The lowest dose of imidazenil producing maximum protection was about 2.5 μmol kg^{-1}; a similar anticonvulsant effect was obtained with 35 μmol kg^{-1} diazepam.

A time-course analysis using the above doses of the tested compounds showed that imidazenil exhibited a longer lasting anticonvulsant action than diazepam (Figure 2). The AUC of the anticonvulsant effects of imidazenil was 1.27 times greater ($P < 0.05$, Student's *t* test) than that of diazepam.

Effect of chronic imidazenil and diazepam on bicuculline-induced convulsions

At the beginning of the chronic experiment imidazenil 2.5 μmol kg^{-1} and diazepam 35 μmol kg^{-1} increased the minimum convulsant dose of bicuculline from 1.32±0.26 μmol kg^{-1} to 4.21±0.40 and 4.38±0.31 μmol kg^{-1}, respectively. A significant reduction in the anticonvulsant efficacy of diazepam was observed in chronic diazepam-treated rats from treatment day 8 (Figure 3). The minimum convulsant dose of bicuculline for groups treated with imidazenil varied between 3.95±0.30 and 4.20±0.24 μmol kg^{-1} during the 130 days treatment (Figure 3), but this did not represent a significant change. On the final day of the chronic diazepam and imidazenil treatment (day 15 and day 130, respectively), the mean minimum convulsant dose of bicuculline in vehicle-treated rats given an acute dose of vehicle, diazepam or imidazenil was not significantly different from that in naive rats on the first day (15 days vehicle-acute vehicle: 1.36±0.23 μmol kg^{-1}; 15 days vehicle-acute diazepam: 4.41±0.38 μmol kg^{-1}; 130 days vehicle-acute vehicle: 1.28±0.28 μmol kg^{-1}; 130 days vehicle-acute imidazenil 4.23±0.36 μmol kg^{-1}).

Plasma concentration of imidazenil and diazepam

Rats chronically treated with imidazenil (130 days), diazepam (15 days) or vehicle and subsequently left drug-free for 18 h were given a single dose of imidazenil (2.5 μmol kg^{-1}) or diazepam (35 μmol kg^{-1}). Plasma samples were collected 30 min later and the concentration of drugs were determined

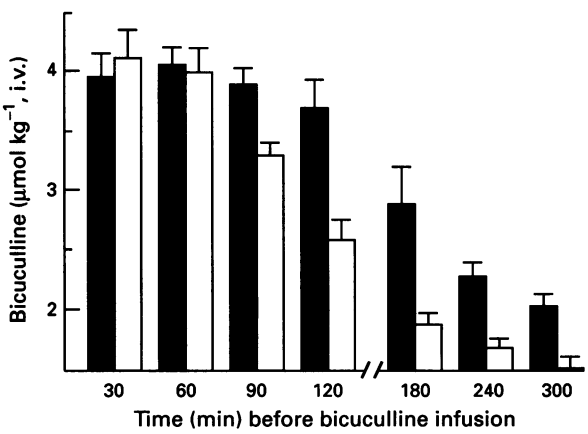

Figure 2 Time-course of anticonvulsant activity of imidazenil (2.5 μmol kg^{-1}, ■) and diazepam (35 μmol kg^{-1}, □). Each point represents the means±s.e.mean of the minimum convulsant dose of bicuculline in 5 rats. Anticonvulsant AUC values for imidazenil and diazepam were 837.8±44.5 and 659.4±35.55 μmol min kg^{-1}, respectively. For experimental details, see Methods.

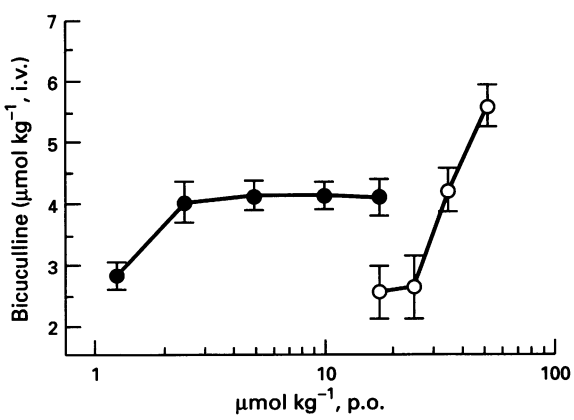

Figure 1 Antagonism by imidazenil (●) and diazepam (○) of bicuculline-induced convulsions. Imidazenil and diazepam were administered 30 min before bicuculline infusion. Each point represents mean±s.e.mean of the minimum convulsant dose of bicuculline in 8 rats. All reported doses of imidazenil and diazepam produced a significant ($P < 0.05$, Dunnett's test) increase in the minimum convulsant dose of bicuculline compared to vehicle-treated rats. For experimental details, see Methods.

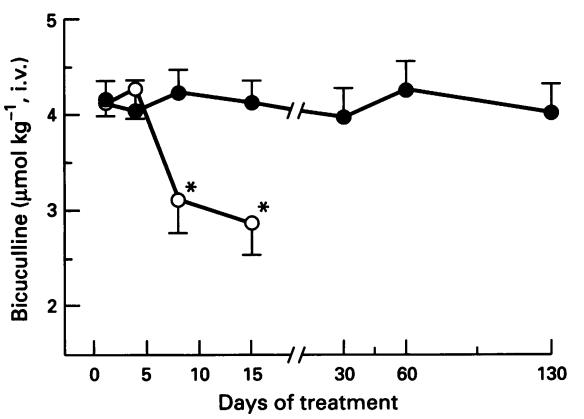

Figure 3 Antagonism by imidazenil (2.5 μmol kg^{-1}) and diazepam (35 μmol kg^{-1}) of bicuculline-induced convulsions in rats chronically treated with imidazenil (2.5 μmol kg^{-1} 3 times a day, ●) and diazepam (35 μmol kg^{-1} 3 times a day, ○), respectively. Imidazenil and diazepam were given 30 min before bicuculline infusion. Each point represents the mean±s.e.mean of the minimum convulsant dose of bicuculline in 5 rats. *$P < 0.05$ compared to diazepam-treated rats at day 0. (Dunnett's test). For experimental details, see Methods.

(Table 1). For both imidazenil and diazepam, the level of drugs in plasma were similar in chronic drug- and chronic vehicle-treated animals.

[^{3}H]-flumazenil binding in vitro

Saturation isotherm from both cortex and cerebellum of imidazenil-treated rats showed no difference in [^{3}H]-flumazenil maximum binding sites (B_{max}) (Figure 4 and Table 2). In contrast, a significant decrease in B_{max} was measured in the two brain areas taken from diazepam-treated animals. Though not statistically significant, higher equilibrium dissociation constant (K_d) values were observed in both cortex and cerebellum of rats treated with either drug.

[^{3}H]-flumazenil binding in vivo

The relationship between the fractional occupancy by [^{3}H]-flumazenil of high affinity recognition sites for BZDs located on $GABA_A$ receptors and the dose of imidazenil and diazepam is shown in Figure 5. Table 3 reports the ID_{50}, the slope factor, the specific and non-specific binding for the displacement of [^{3}H]-flumazenil in the same rats. At the concentration of [^{3}H]-flumazenil used the specific binding was significantly decreased in chronic diazepam-treated rats. Moreover, the ID_{50} was significantly increased after chronic diazepam. On the other hand, chronic imidazenil did not modify either [^{3}H]-flumazenil specific binding or the ID_{50}.

Table 1 Plasma concentrations of imidazenil and diazepam 30 min after p.o. administration in chronically treated animals

Treatment	*Imidazenil* (ng ml^{-1})	*Diazepam* (μg ml^{-1})
Vehicle 130-days	113±10	–
Imidazenil 130-days	112±16	–
Vehicle 15-days	–	8.2±0.082
Diazepam 15-days	–	8.3±0.12

Data are means±s.e.mean of four animals per group. For experimental details, see Methods.

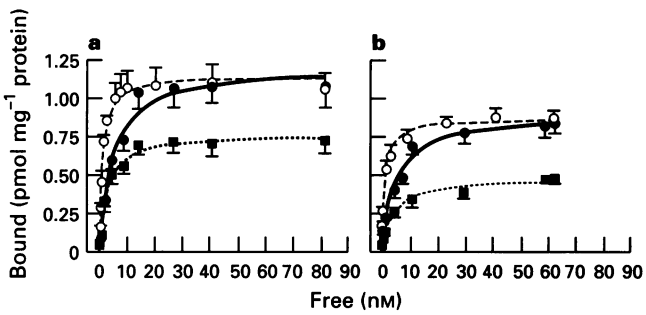

Figure 4 Binding of [^{3}H]-flumazenil (0.1 to 90nM) to (a) cortical and (b) cerebellar membranes from chronic vehicle-treated rats (○), chronic imidazenil-treated rats (●) and chronic diazepam-treated rats (■). Each point represents the mean±s.e.mean from three observations. For experimental details, see Methods. The values of the K_d and B_{max} are given in Table 2.

Discussion

The results indicate that imidazenil, a new positive modulator of GABA function at the BZD receptor, has markedly different effects during chronic administration compared with diazepam, a conventional BZD. First, no tolerance to the anticonvulsant effect of imidazenil was observed over a prolonged period of chronic administration. Second, chronic administration of imidazenil did not produce substantial alterations in BZD receptor density, either *in vitro* or *in vivo*.

In general, BZD receptor down-regulation after chronic administration with BZDs involves a decrease in number of sites rather than a decrease in apparent affinity (Grimm & Hershkovtiz, 1981; Rosenberg & Chiu, 1981; Miller *et al.*, 1988). Although not statistically significant, the increase in K_d values we found in the *in vitro* binding experiments may be due to carry over of interfering concentrations of imidazenil and diazepam in the assay. Consistently, a residual amount of diazepam in the brain may explain the increase in the *in vivo* ID_{50} in the diazepam tolerant rats, although no similar change

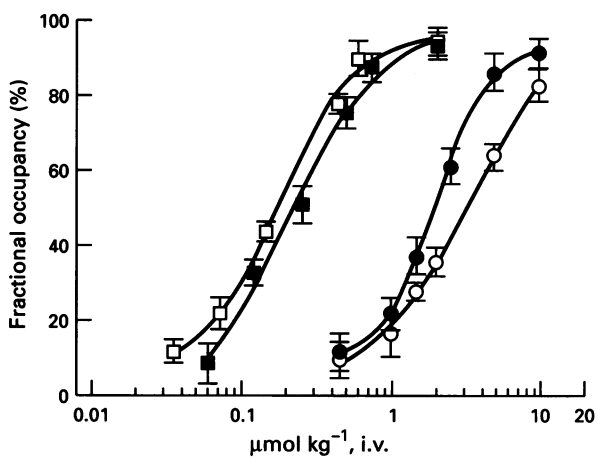

Figure 5 *In vivo* displacement from rat forebrain of [^{3}H]-flumazenil by imidazenil and diazepam in rats treated chronically with imidazenil, diazepam and vehicle ((□) imidazenil in chronic imidazenil-treated rats; (■) imidazenil in chronic vehicle-treated rats; (○) diazepam in chronic diazepam-treated rats; (●) diazepam in chronic vehicle-treated rats). Each point represents the mean± s.e.mean from three observations. For experimental details, see Methods.

Table 2 Effect of chronic administration with imidazenil and diazepam on [^{3}H]-flumazenil binding to rat brain membranes

	Cortex		*Cerebellum*	
Treatment	B_{max} (pmol mg^{-1} protein)	K_d (nM)	B_{max} (pmol mg^{-1} protein)	K_d (nM)
Vehicle	1.20±0.022	1.1±0.29	0.89±0.12	0.89±0.29
Imidazenil	1.20±0.047	4.7±1.54	0.90±0.16	6.1±1.88
Diazepam	0.76±0.017**	2.7±0.65	0.51±0.06*	4.3±1.22

Data are means±s.e.mean of three animals per group. For experimental details, see Methods. *$P<0.05$ and **$P<0.01$ compared to the vehicle, Student's t test.

Table 3 Effect of chronic administration with imidazenil and diazepam on *in vivo* [^{3}H]-flumazenil binding in rat forebrain

Treatment	*Specific binding* (d.p.m. g^{-1})	*ID_{50}* (μmol kg^{-1})	*Slope factor*	*Non-specific binding* (% of total binding)
Acute imidazenil	7880 ± 450	0.18 ± 0.071	1.3 ± 0.54	11
Chronic imidazenil	7690 ± 430	0.18 ± 0.023	1.8 ± 0.34	9
Acute diazepam	7835 ± 650	2.0 ± 0.06	2.5 ± 0.19	11
Chronic diazepam	6025 ± 360*	3.3 ± 0.52*	1.4 ± 0.25	14

Acute imidazenil and acute diazepam denote chronic vehicle-treated rats receiving different doses of these BZD receptor ligands i.v. 10 min before they were killed. Chronic imidazenil and chronic diazepam refer to chronic imidazenil- and chronic diazepam-treated rats injected i.v. with different doses of the two compounds 10 min before they were killed. [^{3}H]-flumazenil was given i.v. to all animals 3 min prior to sacrifice. Data are means ± s.e.mean of three animals per group. For experimental details, see Methods.
*$P < 0.05$ compared to acute diazepam, Student's t test.

was observed for the *in vivo* ID_{50} in the imidazenil treated rats. On the other hand, the lack of change of *in vivo* ID_{50} in imidazenil-treated rats does not apparently agree with the *in vitro* data.

The behavioral findings reported here extend previous observations in mice (Ghiani *et al.*, 1994) and rats (Auta *et al.*, 1994). These authors, in fact, found no tolerance to the anticonvulsant effect of imidazenil after either 14 or 30 days of chronic administration. Furthermore, Ghiani *et al.* (1994) showed no down regulation of $GABA_A$ receptor function as indicated by lack of changes in [^{35}S]-TBPS binding and muscimol-stimulated Cl^- uptake in cerebral cortical membranes. Decreases in GABA- or muscimol-stimulated Cl^- flux and increased [^{35}S]-TBPS binding were instead reported in mice and rats treated with diazepam (Lewin *et al.*, 1989; Marley and Gallagher, 1989; Serra *et al.*, 1994b).

The results obtained with imidazenil in this study are consistent with the partial agonist profile of this compound (Guisti *et al.*, 1993). Studies on GABA-induced membrane conductance in cultured spinal neurones demonstrated that some of the conventional BZDs such as clonazepam and chlorodiazepoxide might have partial agonist properties and this would correlate with the longer duration of treatment required with these two compounds to induce anticonvulsant tolerance (Gent *et al.*, 1985; Garratt *et al.*, 1988). No development of tolerance as well as lack of receptor down regulation and decrease in $GABA_A$ receptor function after chronic administration with partial BZD agonists have been repeatedly reported (Boast & Gerhardt, 1987; Haigh & Feely, 1988b; Feely *et al.*, 1989; Miller *et al.*, 1990). As a general rule, development of tolerance is dependent upon the choice of both dose and duration of administration. Treatments with BZD ligands in the aforementioned studies did not usually extend beyond 30 days. Therefore, it remained possible, that, in light of their low intrinsic activity, development of tolerance to the effect of partial agonists had been obscured by an insufficient duration of the chronic treatment. This hypothesis, however, does not seem to hold true, at least for imidazenil, because of the extensive exposure to the drug in this study. Furthermore, different tolerance liabilities cannot be explained by insufficient exposure to imidazenil as compared to diazepam following each single drug administration. In fact, the opposite was found. AUCs of anticonvulsant activity argued for a longer exposure to the drug after imidazenil administration. Lack of changes in plasma levels of the two drugs after chronic administration in spite of their divergent behavioral outcomes does not support a role for pharmacokinetic factors. Thus, tolerance and lack of tolerance do not seem to be pharmacokinetic in origin. Consistently, the possibility of a metabolic basis for anticonvulsant tolerance has been largely dismissed on evidence from both animals and clinical studies (Haigh & Feely, 1988a). As to the dose factor, whether chronic administration with doses of imidazenil higher than 2.5 μmol kg^{-1}, which is about the most effective dose protecting from seizures in our model, will ultimately induce tolerance is still a possibility to explore. The interaction of imidazenil with BZD recognition sites on the $GABA_A$ receptor complex has been recently investigated (Lipartiti *et al.*, 1995). The thermodynamic analysis of this interaction indicated that imidazenil behaves like an antagonist at the BZD receptor, though it exhibits a positive, albeit partial, modulatory action of GABA function (Giusti *et al.*, 1993; Serra *et al.*, 1994a). The relevance of this finding to the low tolerance potential of imidazenil is unknown at present. Interestingly, Hernandez *et al.* (1989) showed that a 3-week continuous administration of the BZD receptor antagonist, Ro 15-1788 did not produce any change in various measures of GABA sensitivity (i.e. neuronal response to iontophoretically applied GABA, GABA-induced increase in [^{3}H]-flunitrazepam binding, [^{3}H]-bicuculline binding), the alterations of which could be correlated with increasing potential for anticonvulsant tolerance of BZDs.

In conclusion, our results indicate that imidazenil produces neither behavioral tolerance nor substantial receptor down regulation during long-term administration. Should the finding with imidazenil be reproduced at a clinical level during prolonged anticonvulsant therapy, then it may play an important role in the treatment of epilepsy, particularly if it also displays less sedative/muscle relaxant activity. Indeed, imidazenil has not yet been evaluated in human epilepsy. Being a partial agonist, i.e. endowed with less intrinsic activity than a full agonist, one could speculate about the clinical efficacy of imidazenil, and other partial agonists as well. However, the well-established therapeutic use of clonazepam, which also exhibits features of a partial agonist in preclinical models of antiepileptic activity (Haefely *et al.*, 1990), leaves open the possibility that imidazenil may also prove effective in the clinical setting as an antiepileptic agent.

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