

Further pharmacological characterization of eltoprazine: focus on its anxiolytic, anorexic, and adverse-effect potential

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Eltoprazine, a drug that had previously been developed for aggression, has recently been investigated for L-DOPA-induced dyskinesia in animal models of Parkinson's disease (PD) and in dyskinetic PD patients. Much less is known about effects of eltoprazine in other therapeutic indications. Indeed, the pharmacological profile of eltoprazine might suggest its effects on anxiety and food intake, but also adverse effect potential, which is the focus of the present study.

Given for 2 weeks either as infusion or as twice-daily treatment, eltoprazine produced a decrease in food intake and body weight at doses leading to 200–500 nM plasma concentrations.

In the elevated plus maze eltoprazine increased anxiety-like behavior. On the other hand, it induced a clear-cut anxiolytic effect in context fear conditioning test starting at ca. 0.3 mg/kg, and failed to produce any significant effect in fear potentiated startle test.

Regarding adverse effects, eltoprazine was found to produce hypothermia starting from 1 mg/kg. At similar doses it also increased locomotion in the open field. However, eltoprazine failed to affect acquisition in context fear conditioning paradigm, which may indicate lack of its detrimental effect on learning at the doses tested (i.e., up to 5 mg/kg).

In summary, effects of eltoprazine in different anxiety tests were equivocal while its effect on body weight seems robust and requires further investigation. It is to be determined whether these effects can be expected at the doses free of adverse effects.

Key words: anxiety, obesity, adverse-effects, learning, PK

INTRODUCTION

Eltoprazine (1-(2,3-dihydro-1,4-benzodioxin-8-yl)piperazine) is a compound with a plethora of activities at serotonergic (5-HT) receptors, which has recently been developed for L-DOPA-induced dyskinesia in Parkinson's disease. Eltoprazine is a partial agonist at 5-HT_{1A} and 5-HT_{1B} receptors (with different intrinsic activities), a full agonist at 5-HT_{2A} receptors, a partial agonist at 5-HT_{2B} receptors and a full agonist at 5-HT_{2C} receptors (Joels et al. 1990, Schipper et al. 1990). In turn, it can be supposed that the drug may have several potential therapeutic applications.

Eltoprazine was originally developed by Duphar and later by Solvay Pharmaceuticals for the treatment of aggression (Olivier et al. 1990) since it showed clear anti-aggressive effects in dominant behavior assessment in rat colony as well as in animal models such as social interaction or predatory behavior (Olivier et al. 1990). Interestingly, in place conditioning, eltoprazine produced place aversion effect that was attenuated by mCPP (5-HT_{1C} agonist) suggesting

that low intrinsic activity of eltoprazine at 5-HT_{1C} receptor may produce antagonistic-like effect and contribute to emotional behavior (Rocha et al. 1993). However, 5-HT_{1A} receptors also seem to play a role in aggression, since in intruder aggression test effect of eltoprazine was partially attenuated by the selective antagonist WAY-100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride) (de Boer et al. 1999).

In line with these observations, eltoprazine was shown to reduce aggression in intellectually disabled individuals, however this beneficial effect disappeared in the course of treatment (de Koning et al. 1994).

After Solvay's merger with Abbott, the eltoprazine program was out-licensed to PsychoGenics and the indication "adult ADHD" (attention deficit hyperactivity disorder in adults) was added based on the drug's activity in PsychoGenics' proprietary SmartCube® system, exhibiting a behavioral pattern similar to other stimulant and non-stimulant drugs used in the treatment of ADHD

(<http://www.psychogenics.com/newsletter/SmartCube/repurposing.html>).

Based on that, phase II trial in adult ADHD patients was performed providing encouraging results (<http://www.marketwatch.com/story/amarantus-provides-program-update-on-phase-2b-eltoprazine-for-parkinsons-disease-and-adult-adhd-2014-09-17>).

Eltoprazine also has been found to produce antidyskinetic effects in rats with unilateral lesion to the midbrain dopaminergic system and in MPTP treated macaques with some worsening of antiparkinsonian effect of L-DOPA (Bezard et al. 2013). In turn, PsychoGenics added this indication and performed positive phase II trials (2012, Psychogenics.com). A further study sponsored by Michael J. Fox Foundation and PsychoGenics has recently been published (Svenningsson et al. 2015). Next, PsychoGenics out-licensed eltoprazine to Amarantus that has been developing it further, apparently for all three aforementioned indications; however aggression has been limited to the one occurring in Alzheimer's disease and phase II study results announced in December 2015 indicated positive outcome. Recently, eltoprazine received orphan drug status from FDA for L-DOPA-induced dyskinesia.

Given the spectrum of these indications and eltoprazine's unique pharmacological profile, i.e., its different modes of action at several serotonergic receptors, further indications for the drug can be envisaged. However, it should be stressed that adverse effect potential of eltoprazine has not yet been extensively evaluated.

Moreover, anxiolytic effects of eltoprazine have been described (Sanchez 1997), but not intensively characterized and anorectic potential of substances acting at 5-HT_{2C} receptors has also been shown (Marston and Heisler 2009), albeit not specifically for eltoprazine.

Therefore the present study was aimed at testing anorectic and anxiolytic effects of eltoprazine as compared to adverse effects such as body temperature change, learning impairment and behavioral stimulation in laboratory rodents. The results have been previously been disclosed as a patent application (Valastro).

METHODS

Animals

In majority of experiments male Sprague-Dawley rats weighing 180–200 g were used. In context fear conditioning experiments Sprague-Dawley rats weighing 230–250 g were employed. The animals (317 in total) were kept up to four per cage (Polysulfone type IV, Tecniplast, Italy), with the exception of weight gain study, in a room with controlled temperature (21±1°C) and humidity (60±3%). Light was on

6 a.m. till 6 p.m. Standard laboratory food (Altromin, Lage, Germany) and water were available ad libitum. The study was approved by the local Ethical Committee (Darmstadt, Hessen). The experiments were performed between 9 a.m. and 5 p.m.

Substances

Eltoprazine (1-(2,3-dihydro-1,4-benzodioxin-8-yl)piperazine) synthesized by Merz Pharmaceuticals, Frankfurt, Germany was dissolved in water (vehicle) and injected s.c. or delivered by osmotic pumps.

Weight gain experiments

The animals were either injected twice daily (b.i.d.) with eltoprazine (1 mg/kg) or implanted osmotic pumps (Alzet, 2ML2, USA) delivering 4.5 µl/h leading to a nominal daily dose of 8 mg/kg.

Osmotic ALZET pumps (DURECT Corporation, Cupertino, USA, Model 2ML2; mean pumping rate 4.5 µl/h) were implanted subcutaneously. Briefly, rats were anaesthetized with 10% Metacam (inhalation anesthesia, isofluran/O₂). The upper part of the animal back was shaved and disinfected, and a longitudinal incision (1 cm) of the skin was made with a scalpel to allow the subcutaneous insertion of an osmotic pump. The pump was positioned in the lower portion of the rat back. The incision was closed with three metal clips.

During the study, the animals kept in single cages were weighed daily between 7.00 and 9.00 and the amount of food ingested was assessed on working days at the same time.

Blood samples were withdrawn by means of retrobulbar venous plexus puncture using micro-hematocrit-tubes (Heiland, Hamburg; Germany) and collected into Lithium-Heparin tubes (Microvette® 500, Sarstedt, Nuembrecht, Germany). Subsequently, the samples were centrifuged (3000 rpm) for 10 minutes and plasma was carefully transferred to glass tubes and stored at -18°C until analysis of eltoprazine concentration.

Elevated plus maze (EPM)

The elevated plus maze made from black polypropylene consisted of two open arms (51×10 cm with 1.3 cm high walls), two closed arms (51×10 cm and 40.6 cm high walls) and a central platform (10×10 cm). The maze was elevated 72.4 cm above the floor and a 20 W normal light bulb was placed 33 cm above each of the open arms. In one experiment, red light 20 W bulb was used. Breaks of photo beams located in each arm entry were recorded and

analyzed automatically using an IBM-PC running MED-PC software version IV (Med Associates, St. Albans, Vermont, USA). A camera positioned 2 m above the maze allowed remote observation of rats' behavior.

After 60 min habituation to the room, the animals were injected with eltoprazine or vehicle and 1 h later placed in the center of the maze facing an enclosed arm. Next, entries into closed and open arms, as well as the time spent in each type of arm, was recorded for 5 min.

Contextual fear condition (CFC) – anxiety and learning

For training and testing, rats were placed in a chamber (31×25×31 cm, w×d×h) equipped with a grid floor designed to deliver electric shocks (Coulbourn Instruments; Model H10-11R-TC-SF, Whitehall, USA). The shocks were applied using electric shock generator and a scrambler. Application of the foot shocks and movement detection of the animals were performed using Freeze-Frame software (version 1.62; Actimetrics, Wilmette, Illinois, USA). Test cages were placed in sound-attenuating isolation cubicles (Coulbourn Instruments; Model H10-24T, 64×51×66 cm, w×d×h) equipped with fans (Coulbourn Instruments; Model ACT-130) producing background noise of 63 dB sound pressure level. An additional noise generator produced white noise to achieve overall background noise level of 65 dB. Digital cameras (Panasonic, Secaucus, New Jersey, USA; Model WV-BP334) were mounted on ceiling of test chambers to detect movements of animals.

For conditioning (training), rats were placed into the chambers and following a 2-min habituation, 3 electric foot shocks (0.4 mA, 1 s) were delivered, with a 60-s interval. Thirty seconds later the animals were removed from the chambers.

For testing, 24 h later the rats were placed again in the chambers and their freezing behavior was recorded for a total of 5 min. The percentage of time spent freezing during the 5-min test was used for analysis.

In the version of this test used to measure anxiety, eltoprazine was injected 1 h before test. For testing effect on acquisition of fear memory, the drug was injected 1 h before training.

Fear potentiated startle (FPS)

Apparatus

For training, the subjects were placed in acrylic animal holders (19 cm long, 7.6 cm internal diameter (ID) with a grid floor consisting of 9 stainless steel bars (3 mm ID). The holders were fixed onto a startle platform (Med associates,

Model PHM-250B). The grid floor was connected to an electric shock generator and scrambler, by which a 0.6 mA foot shock was delivered. The complete equipment was placed into a sound-attenuating chamber equipped with the speaker placed 7 cm from the animal holder in the back of the chamber and a fan attached on the side wall producing a background noise of 62 dB (together with background noise generator it was 64 dB). Startle-eliciting noise 50 ms bursts were generated by a noise generator (Med associates, Model PHM-255A). A 3.7 sec visual cue was produced by fluorescent stimulator (Med Associates, Model PHM-258L) using a 8 W bulb, placed in front of the chamber. The output of the accelerometer was connected through an interface to an IBM-PC running Startle Reflex software (Med associates, version 5.1).

Procedure

Pre-test

On the first day, in order to obtain groups with similar startle responses, a pre-test was performed. The subjects were placed into the acrylic holders, and after a 5 min acclimation, 6 initial startle stimuli (white noise, 100 dB, 50 ms duration) were presented to induce a stable startle baseline. Then, each subject received 15 startle stimuli of 100 dB (with 7–23 sec. inter-stimulus interval).

Conditioning

For training 24 h later, the rats were placed back into the animal holders containing the grid floors. After a 5 min acclimation, 15 pairings of light with a 0.6 mA foot shock were presented. The unconditioned stimulus was presented during the last 500 ms of the 3.7 sec of light, so that both stimuli terminated together. The mean inter-trial interval was 2 min with a range of 90–150 sec.

Test

24 h after conditioning, the rats again were placed into the animal holders. After a 5 min acclimation period, the animals received 6 initial noise bursts of 100 dB to establish a stable startle baseline before recording. Then, 24 startle stimuli (50 ms, 100 dB) were presented, half of them 3200 ms after the onset of the light (light-noise trials), and half of them in dark (noise alone trials). Inter-trial interval was 15–45 sec. Differences of light-noise and noise alone trials were calculated.

Body temperature

Body temperature was measured 60 min before injection, and then animals were randomized and

injected s.c. with eltoprazine (0.1, 0.3, 1, or 3 mg/kg,) or saline. Rectal temperature was measured 15, 30, 45 and 60 min after treatment.

Open field

Rats were habituated to the experimental room for 1 h prior to testing. Eltoprazine was administered and the animals were placed in open field boxes 1 h later and locomotor activity was measured in Perspex boxes (43.2×43.2×30 cm) placed in noise-proof chambers with white light (5.6 W) placed 55 cm above the floor (Med-Associates Inc. system, St. Albans, USA). Each chamber was equipped in 16 infrared photo beams 3 cm above the floor to measure horizontal activity. Two sets of 16 photo beams placed 15 cm above the floor level were employed to measure vertical activity. Box size was set to “4” (corresponds to a minimal number of photo beam interruptions resulting in counts) which precluded counting of stereotypy. Recording of beam interruptions was continued for 60 or 120 min in 10- or 20-min intervals (depending on an experiment).

Statistics

All results were expressed as means \pm SEM with exception of plasma levels, which were shown as individual values and their medians. Data were analysed by ANOVA followed by Dunnett’s test or ANOVA on ranks followed by Mann-Whitney test depending on values’ distribution. $P < 0.05$ was regarded as significant.

RESULTS

Weight gain

Both infusion of eltoprazine (8 mg/kg/d) and injections twice daily (1 mg/kg) for 14 days produced sustained inhibition of weight gain (Fig. 1A, ANOVA $F(3,28)=17.0$, $p < 0.001$). The effect size was similar from day 4 to 14. There was trend for stronger effect following the infusion which is likely due to approximately 2 times higher plasma levels achieved after this mode of treatment (Fig. 2). Eltoprazine also affected food intake (Fig. 1B, ANOVA $F(2,28)=11.1$, $p < 0.001$), which can at least partly explain its effect on weight gain.

Anxiety

In the elevated plus maze, eltoprazine surprisingly produced a decrease in time spent in open arms and in a number of open arm entries starting at 0.31 mg/kg (Fig. 3, ANOVA $F(2,28)=6.1$, $P=0.003$ and $F(3,28)=3.9$, $p=0.019$ for time in open arms and entries respectively), while having no effect on total entries. This indicates “anxiogenic-like” effect with no change in general activity. As the bright light in a plus maze test is a major trigger to prevent animals from visiting open arms, we also repeated the test under red light conditions as rats do not perceive red light wavelengths. As expected, the time on open arms and number of visits to open arms were found to be much higher at these conditions (Fig. 4, ANOVA $F(3,36)=5.7$, $p=0.003$ and $F(3,36)=13.9$, $P < 0.001$ for time in open arms and

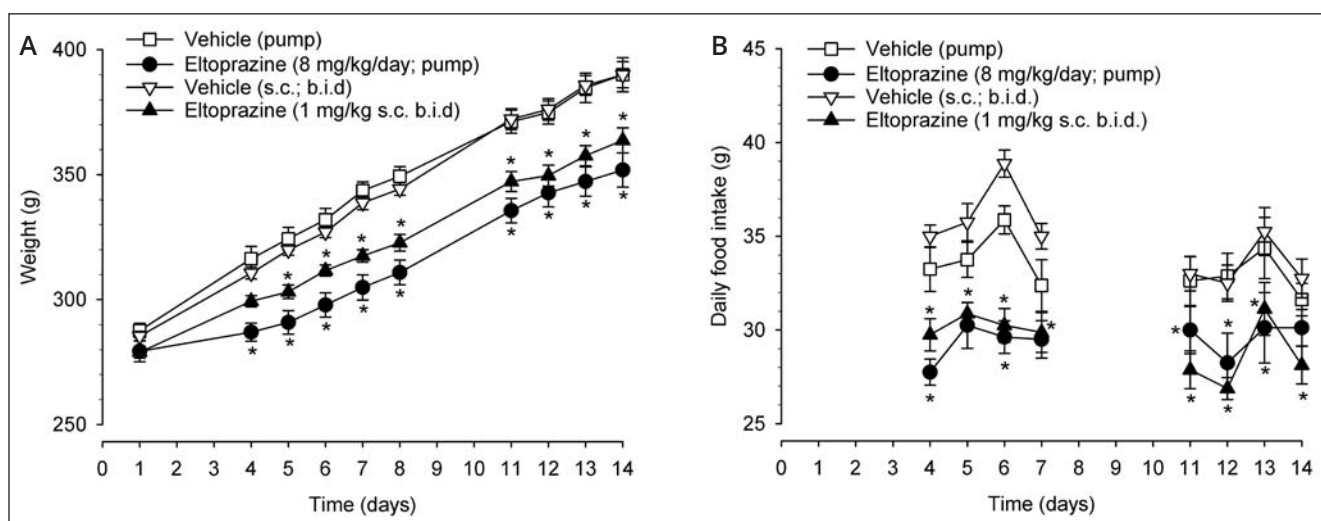


Fig. 1. Effect of eltoprazine on weight gain (A) and food intake (B) in rats. The animals were either injected b.i.d. (1 mg/kg s.c.) or implanted osmotic pumps allowing constant drug delivery (8 mg/kg/day). The values are shown as mean \pm SEM. The data were analyzed using two-way ANOVA with time as repetitive measure, which showed significant effect of treatment, day and their interaction. # - $P < 0.05$ vs. vehicle s.c. injected group; * - $P < 0.05$ vs. vehicle infused group, Dunnett’s *post-hoc* test. $N=8$ per group.

entries respectively). However, eltoprazine still produced anxiogenic-like effect. It should be noted that under red light conditions there was a modest, but significant increase in general activity (total arm entries) following the highest eltoprazine dose of 1 mg/kg (ANOVA $F(3,36)=6.2$, $P=0.002$).

In a further model of anxiety, context fear conditioning, a strong, dose-dependent decrease in the freezing time after eltoprazine was observed, suggesting anxiolytic-like activity (Fig. 5, ANOVA on ranks $H=12.58$ with 3 degrees of freedom, $p=0.006$). A significant effect was achieved already at 0.078 mg/kg.

In contrast, in yet another model of anxiety, fear potentiated startle, eltoprazine failed to produce a significant effect at doses up to 1 mg/kg (Fig. 6, ANOVA $F(3,33)=0.51$, $P=0.68$). The drug also failed to affect acoustic startle reaction alone.

Adverse effects

Eltoprazine at low dose (0.1 mg/kg) produced slight hyperthermia, which was however significant at just one time point (30 min). In contrast, at higher doses, the drug induced dose-dependent hypothermia reaching significance at 1 mg/kg (Fig. 7, ANOVA $F(4,20)=8.1$ $p<0.001$).

In the open field test, eltoprazine produced an increase in activity, which at lower doses was only evident during first recording period (20 min). However at a high dose of 10 mg/kg was significant for the entire observation period, i.e., 120 min (Figs 8A, 8C, ANOVA $F(3,28)=19.28$, $p<0.001$ and $F(3,28)=34.76$, $p<0.001$ for A and C respectively). In parallel, there was a decrease in vertical activity (rearing) in the first observation period after 3 and 10 mg/kg (Figs 8B, 8D,

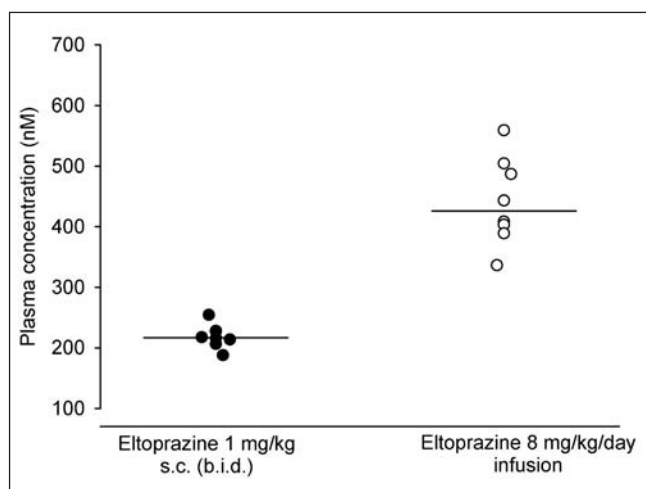


Fig. 2. Plasma concentrations of eltoprazine after repetitive treatment b.i.d (1 mg/kg, s.c., 14 days) or infusion (8 mg/kg/day, 14 days) using osmotic Alzet pumps. Individual values are shown and the horizontal lines represent median values. Animals were sacrificed 1 h after the last injection or directly after pumps' explantation. $N=8$ per group.

ANOVA $F(3,28)=2.2$, $p<0.11$ and $F(3,28)=6.44$, $p=0.002$ for B and D respectively).

Finally, eltoprazine was tested in contextual fear conditioning paradigm allowing testing of learning, i.e.,

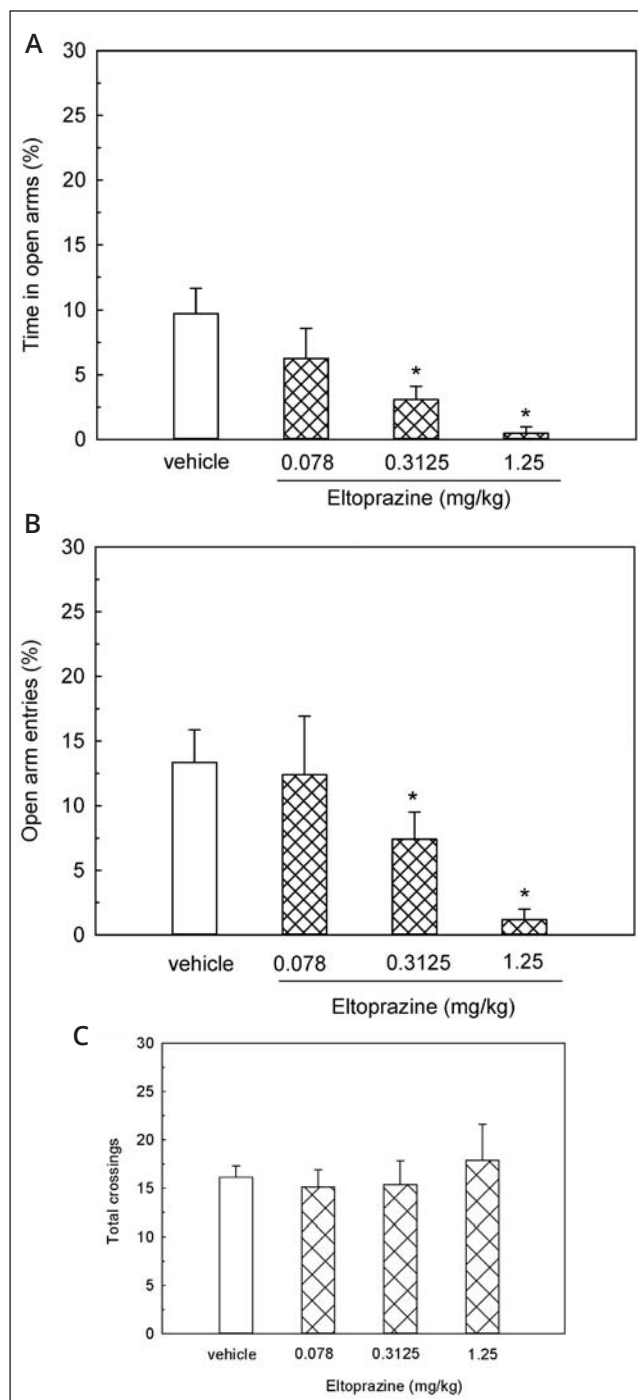


Fig. 3. Effect of eltoprazine (injected s.c. 1 h before the test) on anxiety in elevated plus maze expressed as % time in open arms (A) and % open arm entries (B). Panel C shows total entries as measure of general activity. The values were analyzed using one way ANOVA followed by Dunnett's *post-hoc* test and are shown as mean \pm SEM. * - $P<0.05$ vs. vehicle. $N=8$ per group.

injected before training and tested 24 h later (Fig. 9). In this experimental design, we observed lack of significant effect of eltoprazine on freezing response at doses up to 5 mg/kg as revealed by Kruskal-Wallis test (ANOVA on ranks, $P=0.062$).

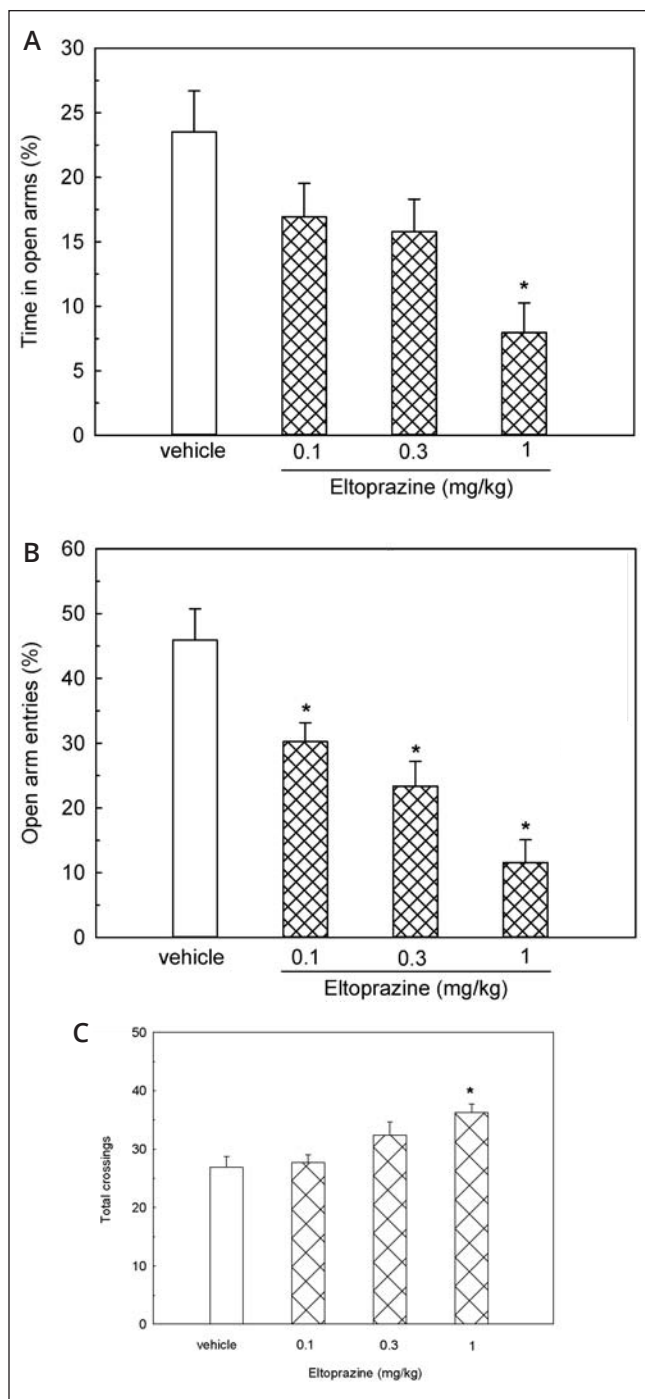


Fig. 4. Effect of eltoprazine (injected s.c. 1 h before the test) on anxiety in elevated plus maze under red light conditions expressed as % time in open arms (A) and % open arm entries (B). Panel C shows total entries as measure of general activity. The values were analyzed using one way ANOVA followed by Dunnett's *post-hoc* test and are shown as mean \pm SEM and. * - $P < 0.05$ vs. vehicle. N=10 per group.

However, on the graph a strong trend for disruption can be seen at the dose of 5 mg/kg. A direct pairwise comparison (Mann-Whitney test) showed significant difference of this group to vehicle confirming that this trend may be an expression of real difference. Nevertheless, the dose of 1.25 and lower can be regards as likely free of this adverse effect (Fig. 9).

DISCUSSION

We observed that eltoprazine given repetitively or infused to rats produced a decrease of body weight of approximately 20–40 g within the study duration (2 weeks). At the same time, there was also a substantial decrease in food intake. To the best of our knowledge, no data have been published on such activity of eltoprazine beyond a patent filed by Merz (<http://www.google.com.tr/patents/US20120190690>). Dose of eltoprazine shown in the present study to affect body weight is similar to, or slightly higher than the dose producing antidykinetic effect (Bezard et al. 2013). It remains to be determined what the minimal threshold dose showing such activity is, as in the present study only one dose per treatment mode was employed. The used treatment regimens i.e. injection of 1 mg/kg and infusion of 8 mg/kg/day produced respectively 220 and 440 nM concentration in plasma. Both treatments produced significant effect on weight gain, slightly stronger in case of infusion. Since infusion better models situation in man, where metabolism is slower and steady-state levels can be achieved this way of treatment is more relevant.

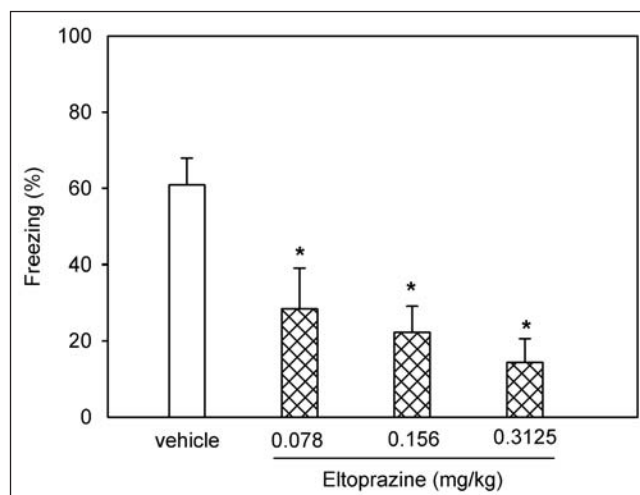


Fig. 5. Effect of eltoprazine (injected s.c.) on anxiety in contextual fear conditioning in rats. The animals were exposed to 5 shocks in the test cage on day 1 and on day 2 they were injected with eltoprazine and placed again in the test cages 1 h later. Freezing was used as a measure of anxiety. The values are shown as mean \pm SEM. The data were analyzed using ANOVA on ranks followed by Mann-Whitney test. * - $P < 0.05$ vs. vehicle. N=8–9 per group.

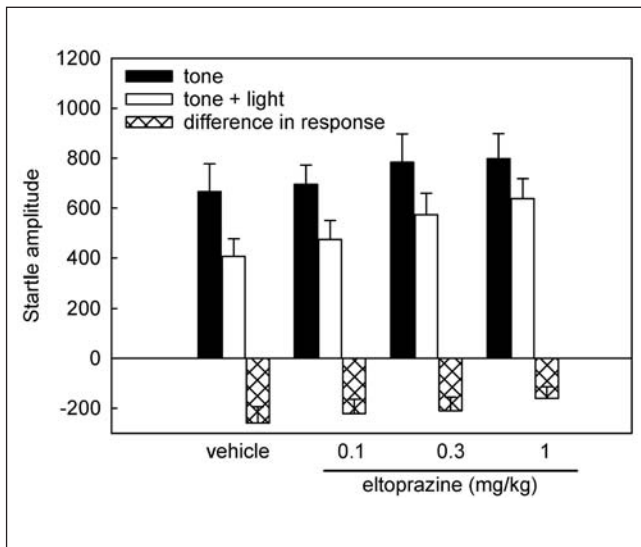


Fig. 6. Effect of eltoprazine on fear-potentiated startle in rats. Etoprazine was injected s.c. 1 h before the test. The values are shown as mean \pm SEM and were analyzed using one-way ANOVA. There was no significant effect of treatment. N=10 per group.

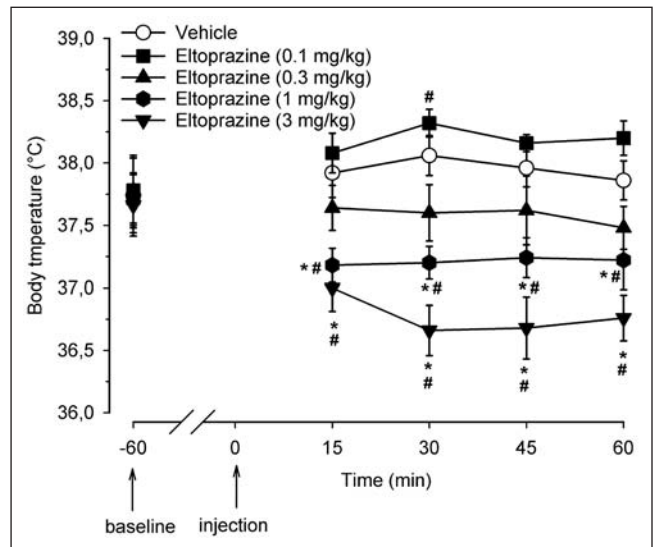


Fig. 7. Effect of eltoprazine on body temperature in rats. Baseline body temperature was measured, 60 min later eltoprazine was injected s.c. and 15 min later temperature measurements were initiated. The values are shown as mean \pm SEM and were analyzed using two-way repeated-measures ANOVA with time as repetitive measure followed by Dunnett's test. * - $P < 0.05$ vs. vehicle; # - $P < 0.05$ vs. respective baseline (-60 min).

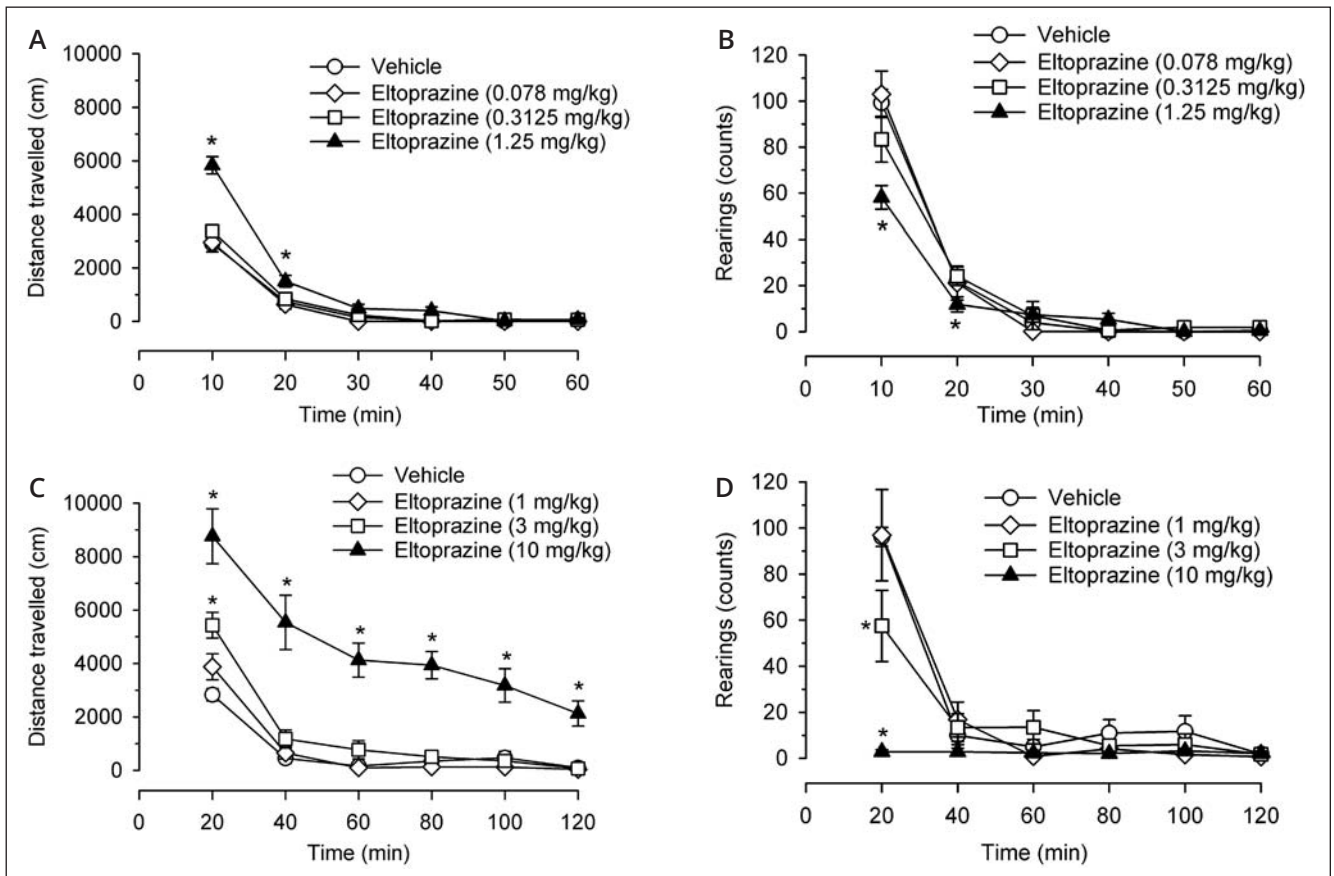


Fig. 8. Effect of eltoprazine at a low dose range (A, B) and a high dose range (C, D) on horizontal locomotor activity (A, C) and vertical activity (rearing; B, D) in rats. The animals were either injected s.c. with eltoprazine and placed in open field 1 h later. The values are shown as mean \pm SEM and were analyzed using two-way ANOVA with time as repetitive measure, followed by Dunnett's *post-hoc* test. * - $P < 0.05$ vs. vehicle. N=8 per group.

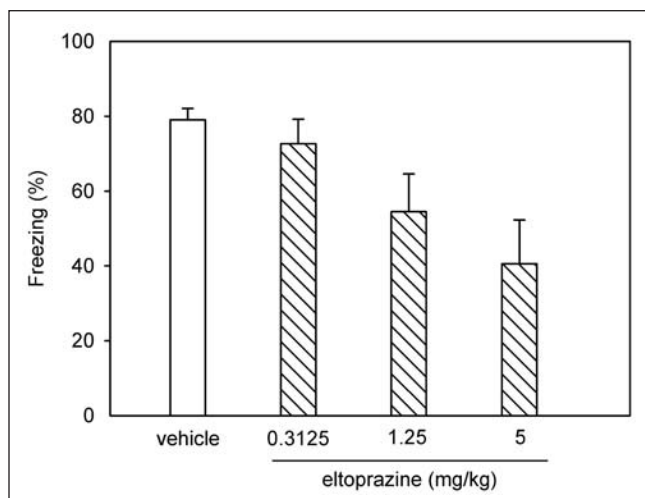


Fig. 9. Effect of eltoprazine on learning in context fear conditioning. The animals were injected with eltoprazine (s.c.) and 1 h later exposed to 5 shocks in the test cage. On the subsequent day the animals were placed in the test cages again. Freezing was used as a measure of retention of the previously learned context fear. The values are shown as mean \pm SEM. The data were analyzed using ANOVA on ranks. N=7–8 per group.

The mechanism of action has not been addressed in the present study. However, given pharmacological profile of eltoprazine, the effect on weight gain and food intake could be related to its agonistic action at 5-HT_{2C} receptors. In fact, therapeutic efficacy of fenfluramine (indirect 5-HT agonist) in obesity has previously been associated with its effect on 5-HT_{2C} receptors (Miller 2005). In fact, this receptor subtype is considered a promising candidate for development of anti-obesity drugs, some of which are under development (Marston and Heisler 2009) and Belviq (Lorcaserin) relatively selective 5-HT_{2C} agonist has been registered by Arena Pharmaceuticals (Brashier et al. 2014).

In the elevated plus maze test we observed a decrease in entries into the open arms and the time spent in the open arms, which may indicate anxiogenic-like activity. In this test, both illumination intensity and lack of walls in two arms contribute to fear and consequently to avoiding open arms. The change of experimental conditions to red light increased as expected time spent in open arms in control animals but has failed to change the effect of eltoprazine indicating that it is not related to, e.g., photophobia. Previously, Rodgers and colleagues (Rodgers et al. 1992) have observed increase in anxiety in the plus maze after eltoprazine, an effect similar to that of fluprazine, TFMPP and mCPP. In contrast, 8-OH-DPAT (5-HT_{1A} agonist) produced anxiolytic effect while CGS 12066B (5-HT_{1B} agonist) produced an anxiogenic response suggesting that 5-HT_{1B} receptors are likely contributors to the anxiogenic action of eltoprazine. However, in another study, chronic treatment with eltoprazine increased anxiety in plus maze and also increased 5-HT_{2C}

receptors density in the amygdala (Rocha et al. 1994) suggesting possible involvement of other subtypes of 5-HT receptors as well.

In contrast to the elevated plus maze data, eltoprazine administered at low doses produced clear-cut anxiolytic-like response in the conditioned fear conditioning test. However it failed to produce any anxiolytic-like effect in a test based on measuring startle response, i.e., fear potentiated startle even at a dose of 1 mg/kg. The reason for this mixed profile in anxiety tests is unclear. Based on results from animal models of anxiety eltoprazine's therapeutic potential for anxiety treatment is rather weak.

Eltoprazine also enhances exploration of the white box in mice (Sanchez 1997). In stress-induced hyperthermia test, eltoprazine failed to demonstrate any effect (Zethof et al. 1995), a finding somewhat contradictory to the results of the present study, where dose-dependent hypothermia was observed. Eltoprazine also abolished stress-induced ultrasonic vocalization in adult rats (Sanchez 1993)

Eltoprazine produced dose-dependent hypothermia with a minimal effective dose of 1 mg/kg, which seems to be within range decreasing weight gain but slightly higher than doses reported to exert antidyskinetic effects (0.3 mg/kg) (Paolone et al. 2015). Hyperthermic activity of 5-HT_{1A} ligands seems to correlate with the potency and the intrinsic activity at the 5-HT_{1A} receptor (Millan et al. 1993).

In the open field test eltoprazine produced locomotor activation starting at 1.25 mg/kg and also a decrease in vertical activity (rearings) which is often related. Similarly to temperature changes, these effects seem to occur at the doses above expected therapeutic range for dyskinesia, but at doses active in weight loss.

Eltoprazine, injected before training of CFC, up to 5 mg/kg did not affect freezing response on the next day in the same context, indicating lack of negative effect on learning acquisition. It should be noted that a strong trend toward impairment was observed which however failed to reach significance. Again, this seems to occur at high doses.

In place conditioning test eltoprazine produced place aversion dependent on 5-HT_{1C} receptor antagonists activity as it was attenuated by an agonist (Rocha et al. 1993).

CONCLUSIONS

Eltoprazine failed to produce consistent anxiolytic activity in animal models but clearly affected weight gain and food intake suggesting potential as anti-obesity substance which however needs further investigation in dedicated animal models in particular definition of minimally effective dose. In turn, it also has to be shown that such effects appear at the doses free of adverse effects such as change in body temperature and locomotor stimulation.

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