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BRIEF COMMUNICATION

Gepirone and 1-(2-Pyrimidinyl)-Piperazine–Induced Reduction of Aversively Evoked Ultrasonic Vocalisation in the Rat

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CULLEN, W. K. AND M. J. ROWAN. *Gepirone and 1-(2-pyrimidinyl)-piperazine-induced reduction of aversively evoked ultrasonic vocalisation in the rat.* PHARMACOL BIOCHEM BEHAV **48**(1) 301-306, 1994.—Ultrasonic (22 kHz) vocalisation in response to a mildly aversive foot shock was measured in the dark compartment of a light-dark box both immediately and 24 h after the shock. Gepirone (1 and 5 mg/kg, IP) produced a reduction in the duration of vocalisation at both times. Although a metabolic inhibitor, proadifen (40 mg/kg) did not reduce this effect of gepirone, the gepirone hepatic metabolite, 1-(2-pyrimidinyl)-piperazine (1-PP, 1 mg/kg), was also active in the test. Performance of a 24 h step-through passive avoidance task was impaired by gepirone only at a dose, 5 mg/kg, which also reduced spontaneous locomotor and rearing activity in the apparatus. It would appear that mild foot shock-evoked ultrasonic vocalisation may provide a more sensitive indicator of the effect of gepirone and related drugs on the affective response of rats to aversive stimulation.

Gepirone 1-(2-Pyrimidinyl)-piperazine Proadifen Ultrasonic vocalisation Passive avoidance Rat

ADULT rats emit ultrasonic vocalisations when subjected to inescapable stressful stimuli such as rough handling (1), mild electric shocks (1,22), or acoustic startle stimuli (10). The emissions usually consist of very loud pure whistles, with a dominant frequency of between 20 and 30 kHz. It has been suggested that calls at approximately 22 kHz signal defensive states of contact avoidance (13) and may provide a quantitative index of the affective response of rats to stressful events and its modification by drugs (7,15,20), although other physiological roles have been suggested (4). Benzodiazepine anxiolytics but not neuroleptics or antidepressants reduce vocalisations induced by mild foot shock (7,11). The recent reports that atypical anxiolytics such as the azapirones buspirone (18) and ipsapirone (11) produced similar suppressant effects provides evidence that adult rat ultrasonic vocalisations may be sensitive to a wider range of anxiolytics than more classical tests (21). Ultrasonic vocalisation in neonatal rats has also been shown to be sensitive to a number of anxiolytic compounds, but has the disadvantage of being highly developmentally dependent (24).

The aim of the present study was to determine whether the buspirone analogue gepirone and the major azapirone hepatic metabolite 1-(2-pyrimidinyl)-piperazine (1-PP,5) affected adult rat foot shock-evoked ultrasonic vocalisation in a manner similar to previously studied anxiolytics. The possible contribution of 1-PP to the effects of gepirone were assessed by pretreatment with the metabolic inhibitor proadifen (SKF 525A). The effects of the drugs on the duration of ultrasonic vocalisation, step-through passive avoidance learning and spontaneous motor activity were compared as part of the same experimental protocol. Passive avoidance has been used in the assessment of anxiolytic (12) and memory (6) effects of 5-HT_{1A} drugs, whereas the spontaneous motor activity measure provided a means of detecting nonspecific effects on behaviour.

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METHOD

Subjects

Male albino Wistar rats (in-house strain from Bio Resources Unit, Trinity College, Dublin), weighing 200 g at the beginning of the experiment, were housed in pairs under a 12 L : 12 D cycle with free access to food and water. The experiments were carried out between 0900 and 1200 h. Each rat was handled for 1–2 min and given a dummy injection with a needle on the two days prior to commencing the experiments to familiarise the rats with handling and injection.

Apparatus

The methods used were similar to those described by Ögren (17) for passive avoidance learning in a light–dark box. The apparatus consisted of a wooden box divided into two identical compartments (each measuring 25 × 25 × 25 cm), which were separated by a wall with an aperture (8 × 8 cm) centred at its base. A door was located here and was opened manually. One side of the box was painted white and designated the light compartment (LC), and the other side was painted black and designated the dark compartment (DC). The perspex roof of the DC was covered with red cellophane for observation purposes. The floor consisted of stainless steel bars (6 mm diameter) spaced 15 mm apart. It was marked with lines dividing each compartment into four sections (12.5 × 12.5 cm). The stressful foot shock stimulus (2 mA) was delivered through the grid floor of the DC from a constant current unscrambled stimulus generator for 5 s. Illumination was provided by overhead fluorescent lights, and a relatively constant background noise was generated by an extractor fan.

The high-frequency calls were picked up with an acoustic transducer (Polaroid Instrument grade, electrostatic transducer, range 20–200 kHz) placed in the centre of the roof of the DC. The input from the transducer was passed through a preamplifier which, in turn, was connected to a band pass filter that rejected any signal outside the frequency range of interest (20–25 kHz). The signal was digitised and analysed using a MacLab system (Analog Digital Instruments).

Procedure

On the training day each rat was placed in a holding area for 10–20 s prior to being placed in the LC, facing away from the open door. The animal was allowed to explore freely for 180 s, during which time its activity was assessed as the number of lines crossed and rears made in each compartment per min and total number of transitions between the compartments. The total time spent in each compartment was also measured. The rat was then removed to the holding area again. In the training session the rat was replaced in the LC facing away from the open door. When the rat entered the DC, the door was shut and the shock was applied. The duration of vocalisation was measured over the 180 s period immediately after the shock (unconditioned vocalisation). On some occasions a rat failed to enter the DC within the 300 s period allocated and, therefore, was not used in the rest of the experiment. Following the recording of the distress calls from the rat a second shock was applied. The rat was then removed and replaced in its home cage. The steel bars were thoroughly cleaned between each training period.

On day 2, retention of the passive avoidance task was determined 24 h after the shock. The rat was placed in the LC facing away from the open door. The latency to enter the DC with all four feet was recorded as the step-through latency. If a rat failed to enter the DC within 300 s it was removed and assigned a score of 300 s. A recording of conditioned vocalisation was made immediately after this by replacing the rat in the DC for 300 s.

Drugs

Gepirone hydrochloride, 1-pyrimidinyl piperazine (1-PP) (both Bristol-Myers Squibb, Evansville) and proadifen (Smith, Kline and French, Welwyn Garden City) were dissolved in distilled water and injected intraperitoneally (IP) in a volume of 1 ml/kg. An equivalent volume of distilled water was administered to control animals. Gepirone and 1-PP were administered 30–35 min prior to testing. The proadifen was given 4.5 h prior to either gepirone or water. The dose of proadifen chosen (40 mg/kg) should greatly inhibit the hepatic microsomal oxidative metabolism of gepirone to 1-PP in rats (16).

Statistics

The locomotor and rearing activity data were subjected to one-way analysis of variance, with post hoc application of Fisher's least significant difference method for individual group comparisons with controls (23). The shock-evoked ultrasonic vocalisation data were subjected to two-way analysis of variance with repeated measures to detect overall drug effects. One-way analysis of variance was used for the comparisons between single experimental groups and controls. Values are presented as the mean ± SEM. Data for recall of the passive avoidance task were analysed using the Mann–Whitney *U*-test. Values for step-through latency are presented as the median.

RESULTS

Ultrasonic Vocalisation

Whereas unshocked rats were never observed to emit ultrasonic calls when allowed to explore the light–dark box, there was marked 22-Hz vocalization both immediately following the mild footshock and 24 h later, when the animals were re-placed in the dark (i.e., shock) compartment in the absence of shock. Since the animals did not vocalize when they were put in the light compartment 24 h after the shock, the 24 h vocalisations were used as an indicant of conditioned stress to the place of aversive stimulation.

When rats received an injection of either water or gepirone (0.5, 1, and 5 mg/kg, IP) 30 min prior to both sessions there was a significant overall treatment effect, $F(3, 46) = 12.22$, $p < 0.01$, Fig. 1). This was due to a significant reduction in vocalisations at 1 and 5 mg/kg both on day 1, $F(1, 22) = 5.95, p < 0.05$, and $F(1, 12) = 41.13, p < 0.01$, respectively, and day 2, $F(1, 22) = 4.74, p < 0.05$, and $F(1, 12) = 14.45$, $p < 0.01$, respectively.

Pretreatment with proadifen (40 mg/kg, IP) prior to gepirone (1 mg/kg, IP) on both day 1 and day 2 did not prevent the inhibitory effect of gepirone on vocalisation duration, $F(1, 10) = 28.03, p < 0.01$, proadifen plus gepirone vs. proadifen plus water, Fig. 2A. This effect was significant on the unconditioned, $F(1, 10) = 13.20, p < 0.01$, and conditioned vocalisation, $F(1, 10) = 19.13, p < 0.01$.

When rats received an injection of either water or 1-PP (1 mg/kg, 1P) 30 min prior to both sessions there was a significant overall treatment effect, $F(1, 10) = 14.94$, $p < 0.01$, Fig. 2B. 1-PP (1 mg/kg, 1P) had a significant effect both on

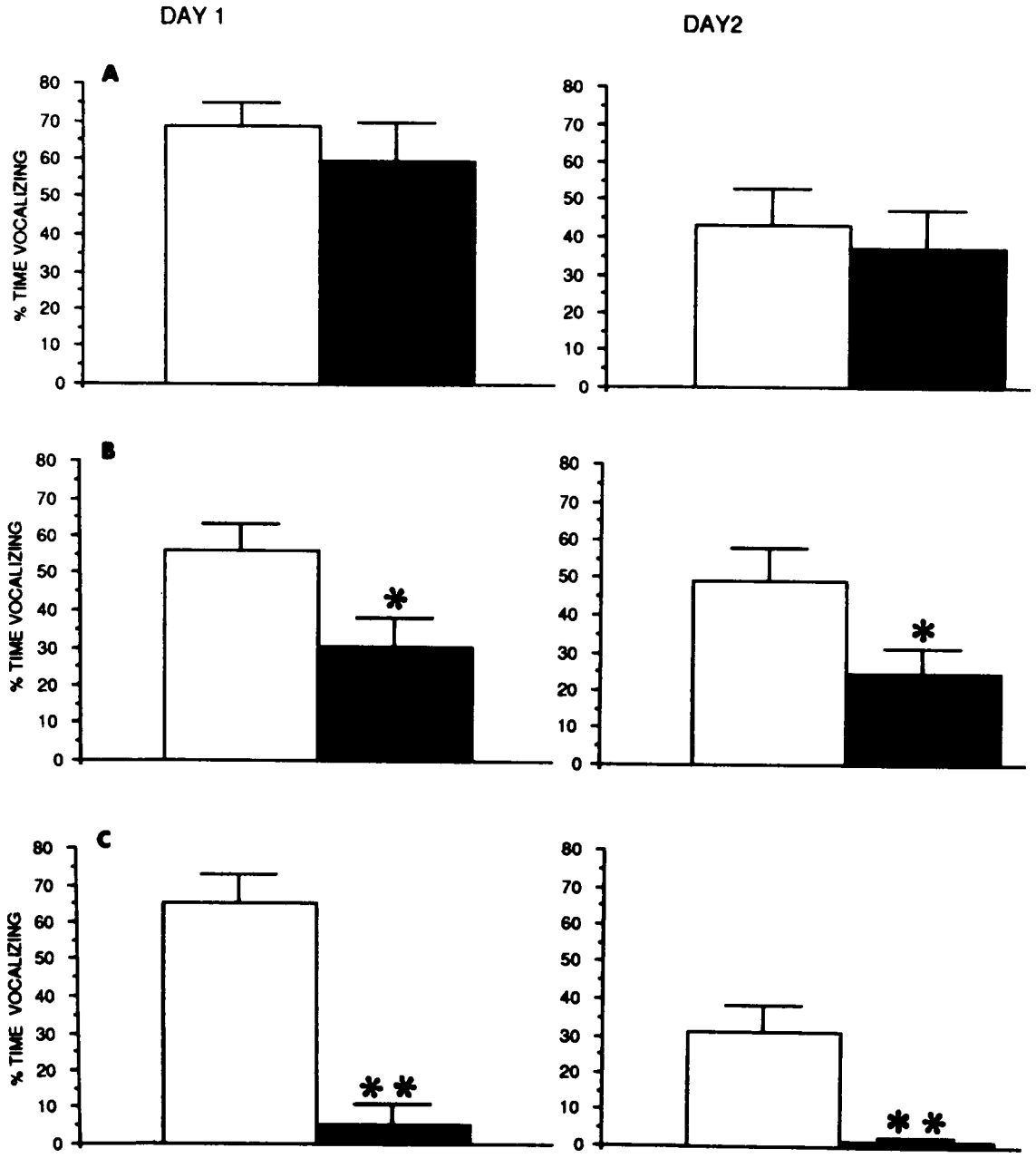

FIG. 1. Effect of gepirone treatment on shock evoked ultrasonic vocalisation in the rat. Water (white bars, n = 7-12) or the drug (black bars) at a dose of 0.5 (A, n = 5), 1 (B, n = 12), and 5 mg/kg, IP (C, n = 7) were injected 30 min prior to recording vocalisation on both days. The vocalisation was monitored either immediately (left hand column, day 1) or 24 h (right hand column, day 2) after foot shock. Values are the mean ± SEM % time spent vocalising. *$p < 0.05$ and **$p < 0.01$ compared to water-injected controls.

day 1, $F(1, 10) = 6.94$, $p < 0.05$, and day 2, $F(1, 10) = 8.05$, $p < 0.01$.

A separate set of experiments were carried out to determine if the effects of gepirone (1 mg/kg) and 1-PP (1 mg/kg, IP) on the conditoned vocalisations were independent of their action on day 1 by administering them 30 min prior to the day 2 session only. There was a significant reduction in the duration of ultrasonic calling in the gepirone-treated animals (0 s, n = 6) compared to water-injected controls (17.3 ± 10 s, n = 6, $p < 0.05$). In 1-PP animals there was a trend towards a significant reduction in the duration of vocalisations [21 ± 8% vs. 44 ± 8%, for water injected controls, $F(1, 10) = 4.06$, $p = 0.07$].

Passive Avoidance Learning

The administration of gepirone (5 mg/kg, IP) prior to both the training and the day 2 test sessions produced a significant

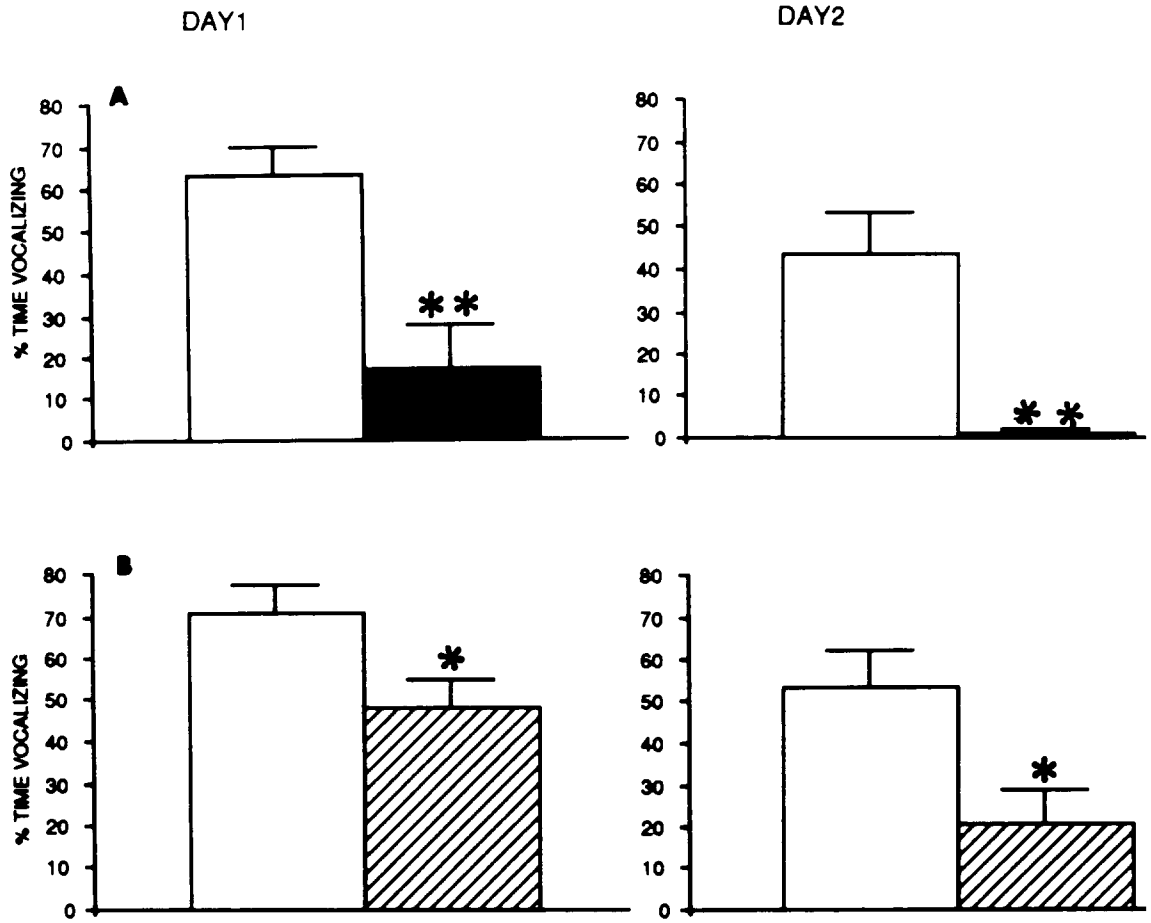

FIG. 2. Effect of (A) gepirone (1 mg/kg, IP) with proadifen (40 mg/kg, IP) and (B) 1-PP (1 mg/kg, IP) on shock evoked ultrasonic vocalisation in the rat. Water (white bars, $n = 6$), gepirone (black bars, $n = 6$), and 1-PP (hatched bars, $n = 6$) were administered 30 min prior to the measurement of vocalisation on both days. In the gepirone study both groups of animals were pretreated with proadifen (40 mg/kg, IP). The vocalisation was monitored either immediately (left hand column, day 1) or 24 h (right hand column, day 2) after the mild foot shock. Values are the mean ± SEM % time spent vocalising. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to water-injected controls.

decrease of step-through latency (median 31.5 s, $n = 7$, compared to 300 s in water-injected controls, $n = 7$, $U = 10.5$, $p < 0.05$). Lower doses when administered prior to both the training session and test session did not produce any statistically significant effect [gepirone 0.5 mg/kg (median 300 s, $n = 5$, compared to 300 s in water-injected controls, $n = 7$, $U = 14$, $p > 0.05$), gepirone 1 mg/kg (median 300s, $n = 12$ compared to 300s in water injected controls, $n = 12$, $U = 63$, $p > 0.05$), and gepirone 2 mg/kg (median 55 s, $n = 7$ compared to 175 s in water-injected controls, $n = 7$, $U = 63, p > 0.05$)].

Pretreatment with proadifen (40 mg/kg, IP) prior to gepirone (1 mg/kg, IP) for both the training and test sessions did not have a statistically significant effect on the animal's performance (median latency of 13.5, $n = 6$, compared to 158 s for animals treated with proadifen plus water, $n = 6$, $U = 14.5, p > 0.05$).

When 1-PP (1 mg/kg, IP) was given 30 min prior to the training and test sessions, it did not have a statistically significant effect (median latency of 12 s, $n = 6$, compared to 190 s for controls, $n = 6$, $U = 10, p > 0.05$).

Light-Dark Box Activity

Gepirone (0, 0.5, 1, 2, and 5 mg/kg; $n = 50$, 6, 12, 16, and 15, respectively) had an overall treatment effect on rearing in the light, $F(4, 94) = 12.49$, $p < 0.01$, and dark compartments, $F(4, 94) = 18.29$, $p < 0.01$. This was due to the effects of the higher doses (2 and 5 mg/kg), the number of rears per minute being less than half the control values ($p < 0.05$). Gepirone also produced an overall treatment effect on the number of lines crossed in the dark compartment, $F(4, 94) = 9.31$, $p < 0.01$. This was also due to the effects of the 2 and 5 mg/kg doses that both decreased the number of lines crossed per minute by approximately 40% ($p < 0.05$).

In proadifen (40 mg/kg; $n = 6$), pretreated animals gepirone (1 mg/kg, IP) had no significant effect on the measures of activity in the light-dark box compared to proadifen plus

water, $F(1, 10) \leq 3.17, p > 0.05$, except for the number of rears per minute in the dark compartment that were decreased by approximately 40%, $F(1, 10) = 6.57, p < 0.05$.

1-PP (1 mg/kg; $n = 6$) had no significant effect on any of the measures of activity in the light–dark box, $F(1, 10) \leq 4.22, p > 0.05$.

DISCUSSION

Gepirone produced a marked reduction in the duration of ultrasonic vocalisations induced by mild foot shock in rats receiving a dose (1 mg/kg) that had no effect on the general activity measures in the light–dark box. This is indicative of a relatively selective antistress effect of the drug at this dose level. The finding that a similar change was observed when the drug was given only on the day 2 test session in the absence of foot shock suggests that an antinociceptive effect was not responsible. A possible amnesic effect cannot be ruled out at 24 h. However, because the drug appeared to be able to affect both unconditioned and conditioned stress responses to a similar degree and because there was no effect of 1 mg/kg gepirone on passive avoidance learning, this would seem unlikely.

Although impairment of performance of passive avoidance tasks is considered a sensitive measure of the anxiolytic effects of 5-HT_{1A} drugs (12), in the present study no consistent decrease in step-through latency was seen until a dose of 5 mg/kg gepirone was administered. This apparent lack of sensitivity of the test to gepirone may be explained partly by the presumably opposing effect that the reduction of activity in the light–dark box, seen at 2 and 5 mg/kg, may have had. Thus, a decrease in exploration would be likely to lead to an increase in step-through latency. This would mask any tendency of the drug to decrease step-through latency as a result of a reduction in the stress associated with the dark compartment. Changes in passive avoidance learning produced by 5-HT_{1A} receptor ligands may also be due to interference with memory (6,18). This further weakens the potential usefulness of this task in assessing the antistress effects of these drugs.

The anxiolytic-like effects of gepirone, buspirone (18), and ipsapirone (1) on ultrasonic vocalisations may be mediated by the shared hepatic metabolite 1-PP. In the present study, strong evidence for a direct involvement of gepirone itself was provided by the observation that the metabolic inhibitor proadifen did not reduce its effect. Indeed, gepirone appeared more potent with regard to changes in ultrasonic calls and rearing activity after proadifen pretreatment. This is in agreement with a report that proadifen augmented the reduction in mouse aggression produced by gepirone (4). In contrast, the alpha 2-adrenoceptor antagonist-like properties of gepirone, which are believed to be due to 1-PP (2), have been shown to be prevented by proadifen (8). Because 1-PP was capable of shortening the duration of vocalisations, its contribution to the effect of the parent compounds cannot be totally excluded. 1-PP has previously been shown to be active in some (9) but not other (25) models of anxiety, whereas it appeared to oppose the effects of 5-HT_{1A} drugs in the learned helplessness model of depression (13). In the present study it appeared to be slightly less effective than gepirone 30 min after administration of a dose of 1 mg/kg. At this time brain concentrations of 1-PP should be much greater after 1-PP injection than after gepirone (5). However, 1-PP accumulates in the brain at much higher levels than its parent compounds (5). It may, thus, play an important role at times when parent compound's levels are relatively low, especially during chronic treatment. Of particular note in this regard, Tollefson et al. (19) have recently found that the clinical anxiolytic effect of buspirone was correlated with plasma concentrations of 1-PP but not of the parent drug after long-term treatment.

In conclusion, foot shock-induced ultrasonic vocalisations in adult rats appear to provide a relatively sensitive quantitative indicator of the antistress effect of gepirone. This effect would appear to be mainly due to the drug itself, even though the major metabolite 1-PP may also have a role.

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