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Hydroxyzine prevents isolation-induced vocalization in guinea pig pups: comparison with chlorpheniramine and immepip

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Abstract

The present pharmacological study was conducted to investigate a possible role of the brain histaminergic system in vocalization induced in guinea pig pups by maternal separation and isolation in an unfamiliar environment. The effects of drugs acting at histamine receptors were determined after intraperitoneal injection, comprising hydroxyzine and chlorpheniramine, both histamine H1 antagonists, and the H3 agonist, immepip. A range of psychoactive drugs known to be active in this paradigm was also tested for comparison. Hydroxyzine, 4.3 to 14.3 mg/kg, dose-dependently suppressed vocalization but neither chlorpheniramine, 2 to 16 mg/kg, nor immepip, 5 to 20 mg/kg, was active. All reference drugs, fluoxetine, 5 and 10 mg/kg, imipramine, 16 and 32 mg/kg, and chlordiazepoxide, 5 and 10 mg/kg, were shown to be active. The present data indicate that, consistent with known anxiolytic effects in man, the antihistamine hydroxyzine proved effective in suppressing maternal-separation-induced vocalization in guinea pig pups. However, in view of the lack of effect of either chlorpheniramine or immepip, it is proposed that additional nonhistaminergic effects are involved in the tranquilizing action of hydroxyzine.

Keywords: Guinea pig pup; Maternal separation; Hydroxyzine; Chlorpheniramine; Immepip; Animal model; Anxiety; Depression; Stress

1. Introduction

Guinea pigs mainly rely on vocal communication to maintain their social group structure (King, 1956). Infant guinea pigs exhibit high rates of vocalization when transiently separated from their mother in an unfamiliar environment (Pettijohn, 1979; Hennessy and Ritchey, 1987). These separation-induced distress calls are mainly audible sounds. Introduction of the mother has been shown to quieten the isolated pup and to suppress the hypothalamo–pituitary–adrenal and sympathetic activation concomitant to this separation (Hennessy and Ritchey, 1987; Hennessy et al., 1989). Moreover, the calls can be reduced by drugs used to treat affective disorders (Molewijk et al., 1996). Most mammals exhibit separation-induced vocalization and the potential application of this phenomenon in laboratory animals as a model of affective disorder has been recognized for at least two decades (Katz, 1981). Whilst separation-induced vocalization in rat pups has indeed been used as a model for screening anxiolytic drugs for over a decade (Gardner, 1985; Insel and Winslow, 1991; Miczek et al., 1995), this paradigm in guinea pig pups was originally studied in relation to development of social bonding (Herman and Panksepp, 1978; Panksepp, 1998). However, recently, interest has been increasing in using separation-induced vocalization in guinea pigs as an animal model to screen drugs that possess potential anxiolytic and/or antidepressant activity (Molewijk et al., 1996; Rupniak et al., 2000; Steinberg et al., 2001). A preference for using this species over the rat is based on several arguments, such as similarities between guinea pig and human receptors, e.g., 5-HT1D or NK1, and importantly, the fact that in contrast to rat or mice pups, guinea pig pups are relatively mature at

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birth. Therefore, physiological/pharmacological studies in pups of this species may be more confidently extrapolated to adults (Ibuka, 1984; Molewijk et al., 1996).

Brain histaminergic systems are known to regulate important integrative nervous system functions, like food intake, fluid balance, temperature regulation and arousal (Brown et al., 2001). Moreover, several studies support a regulatory role for brain histaminergic systems in stress, particularly in the neuroendocrine regulation of stress hormones (Knigge and Warberg, 1991), which has implications for anxiety or even depression (see Brown et al., 2001; Ito, 2000 for review). Postsynaptic H1 and presynaptic H3 receptors are believed to play a key role in this regulation (Soe-Jensen et al., 1993; Yanai et al., 1995; Westerink et al., 2002). Thus, histaminergic neurotransmission via H1 and/or H3 receptors is theoretically an obvious target mechanism to modulate transient maternal-separation-induced HPA axis activation and distress calls in guinea pig pups.

To our knowledge, there are no published findings on the consequence of brain histaminergic modulation on transient maternal-separation-induced vocalization in guinea pig pups. Thus, the present study was aimed at exploring the effect of selected H1 and H3 ligands in this paradigm. The following histamine ligands were evaluated: hydroxyzine (ATARAX), an H1 antagonist, which has been widely used for its tranquilizing and anxiolytic activity in premedication for surgery and anxiety disorders (Argyropoulos et al., 2000; Bandelow et al., 2002; Khalid-Khan et al., 2002); chlorpheniramine, a prototypical selective H1 antagonist (Nicholson et al., 1991) and immepip, a brain-penetrating and selective H3 agonist (Jansen et al., 1998; Lamberty et al., 2003). In addition, imipramine, fluoxetine and chlordiazepoxide, which are all reported to be active in this paradigm (Rupniak et al., 2000; Steinberg et al., 2001), were included, to validate the testing conditions.

2. Materials and methods

2.1. Animal housing

Dunkin Hartley guinea pig dams with a litter of at least three pups aged approximately 24 h old, were purchased from Harlan. On arrival, each guinea pig dam with litter was assigned to an open-topped plastic cage (38×54×29 cm high) with solid floor. Wood shavings were provided as bedding material and sterile shredded paper was provided for nesting. Standard guinea pig diet and tap water were freely available. Guinea pigs remained in their maternal groups throughout the study.

2.2. Procedure

All vocalization testing was conducted between 0900 and 1400 h in a quiet testing room, under normal lighting. The procedure was based on that described by Rupniak et al. (2000). Pretest selection: at approximately 14 days (±2 days) of age, all pups were subjected to pretest screening for isolation-induced vocalization. The pups were transferred individually to the testing room and placed in an empty arena (46×33×25 cm high); the arena was wiped with disinfectant and dried after each pup. The duration of audible vocalization was recorded over a period of 12 min, manually using a stopwatch with the button depressed for the length of each vocalization and also onto magnetic tape via a mounted microphone. The tape recording was made as a contingency measure and to allow assessment of interobserver reproducibility. Only pups which vocalized for a total of at least 5 min were included in the study. The pups were returned to their litter after testing.

Starting at 48 h after the preselection screening, the animals were treated with the compounds and returned to the maternal group for the 30-min dose-test period. The animals were then transferred to the testing room and placed in the arena for audible vocalization recording during a period of 12 min, as described for the preselection test. Gross behavior was also scored, subjectively, on the basis of behavior in the arena during the recording session and response to handling, according to the following system: Score 1: reduced activity with signs of sedation and/or muscle relaxation; Score 2: reduced activity; Score 3: normal; Score 4: increased activity; Score 5: increased activity with signs of excitation. Pups were assigned to treatment groups on a semirandom basis so that each drug group contained no more than one pup from each litter to control for possible litter bias effects. In addition, care was taken prior to drug testing, to ensure that the testing groups were homogeneous with respect to the pretest level of separation-induced vocalization.

The methods used conform to the European Community Council Directive of 24 November 1986 (86/609/EEC) and were approved by our local Ethics Committee for use of experimental animals according to Belgian law.

2.3. Injection of drugs

Hydroxyzine hydrochloride (UCB Pharma), chlorpheniramine maleate (Sigma), immepip dihydrobromide (Tocris), imipramine hydrochloride (Sigma), fluoxetine hydrochloride (Tocris) and chlordiazepoxide hydrochloride (Sigma) were dissolved in 0.9% saline solution. They were injected as a single intraperitoneal injection in a dose volume of 5 ml/kg, 30 min before testing. All doses were expressed in terms of the salt. Control animals received an equivalent volume of saline solution. Treatment groups of at least six animals were used, with no more than one pup from each litter represented in each group.

2.4. Statistical analysis

A one-way analysis of variance (ANOVA) was performed on the data for the increasing dose levels of the

drugs (between-subject design). In case of an overall significant effect, post hoc Dunnett tests were performed for multiple comparisons in regards to controls.

3. Results

As shown in Fig. 1, hydroxyzine, 4.3 to 14.3 mg/kg (corresponding to 10^{-5}, 1.8 and 3.2×10^{-5} mol/kg) dose-dependently decreased the duration of vocalization [ANOVA, $F(3,20)=6.21$, $P<0.005$]; the dose of 14.3 mg/kg was found to be significantly different from control animals (Dunnett test, $P<0.01$). In contrast, neither chlorpheniramine, 2 to 16 mg/kg, nor immepip, 5 to 20 mg/kg, had any significant effect on maternal-separation-induced vocalization in pups [ANOVA, $F(4,37)=0.69$, $P>0.05$ and $F(3,20)=0.45$, $P>0.05$, respectively].

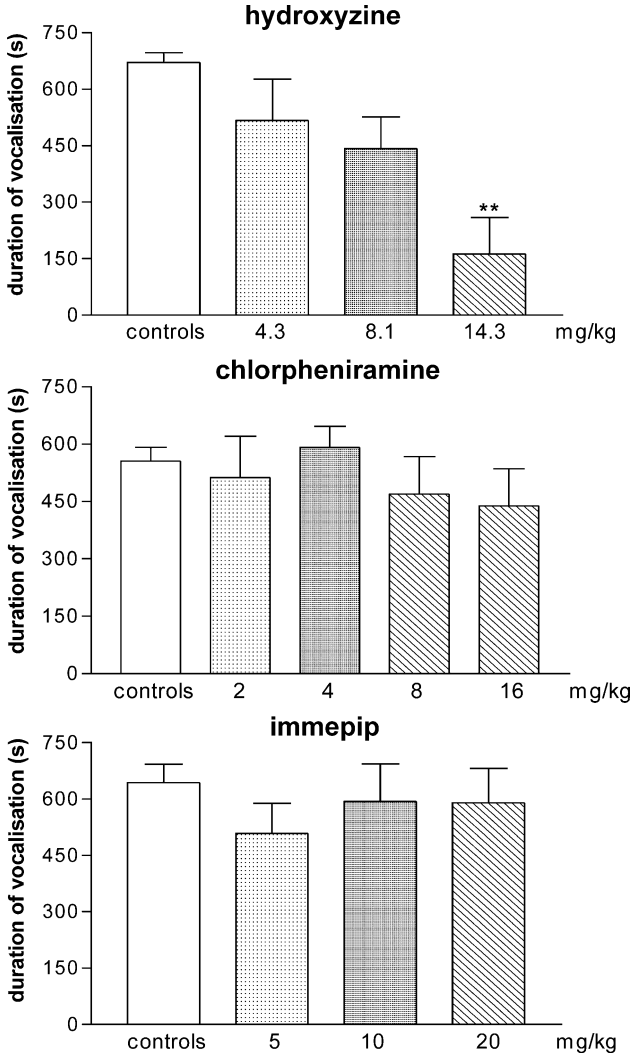

Fig. 1. Mean duration of vocalization by guinea pig pups isolated from their mother in a novel cage for 12 min. Guinea pig pups were injected intraperitoneally with hydroxyzine, chlorpheniramine or immepip, 30 min before testing. Vertical lines represent standard error of the mean. ** $P<0.01$, relative to controls (post hoc Dunnett test).

Table 1
Effect of reference drugs on separation-induced vocalization in guinea pig pups

Compound (mg/kg)	Duration of vocalization (s)
Fluoxetine	
0	633 (24)
5	362 (83)
10	195 (94)**
Imipramine	
0	548 (48)
16	317 (81)
32	85 (84)**
Chlordiazepoxide	
0	670 (25)
5	492 (102)
10	343 (89)**

Results represent mean duration of vocalization of guinea pig pups isolated in a novel test cage during a period of 12 min. The values in parentheses are the standard errors of the mean.
** $P<0.01$, relative to controls (Dose 0; post hoc Dunnett test).

ANOVA indicated that administration of imipramine, 16 and 32 mg/kg, fluoxetine, 5 and 10 mg/kg and chlordiazepoxide, 5 and 10 mg/kg (Table 1), resulted in a dose-dependent and statistically significant overall decrease in vocalization [$F(2,20)=9.29$, $P<0.005$; $F(2,26)=8.21$, $P<0.005$ and $F(2,21)=7.37$, $P<0.01$, respectively]. Imipramine 32 mg/kg, fluoxetine 10 mg/kg and chlordiazepoxide 10 mg/kg induced a statistically significant effect relative to control animals (Dunnett test, $P<0.01$).

Subjective evaluation of gross behavior indicated that all drugs tested induced decreased motor activity at doses that produced statistically significant effects. The highest doses of fluoxetine, hydroxyzine, chlorpheniramine and immepip were attributed a median score of 2. As for imipramine and chlordiazepoxide, both drugs decreased motor activity at the highest doses tested with a median score of 1.

4. Discussion

The present study was conducted to investigate a possible role of the histaminergic system in separation-induced vocalization in guinea pig pups by determining the effects of drugs acting at histamine receptors. The main finding was that hydroxyzine dose-dependently suppressed vocalization but neither chlorpheniramine nor immepip was active. These contrasting effects appear to suggest that additional nonhistaminergic actions may be involved in the observed activity of hydroxyzine.

The positive result of hydroxyzine contrasting with the lack of effect of chlorpheniramine is intriguing. Both drugs are potent and brain-penetrating H1 antagonists which have been reported to be equipotent in their occupancy of brain H1 receptors (Snyder and Snowman, 1987). Chlorpheniramine was inactive at doses far exceeding those known to

block H1 receptors in the guinea pig brain. For example, a dosage of 2 mg/kg has been shown to block over 80% of H1 receptors (Yanai et al., 1995), although the drug was completely inactive, tested up to 16 mg/kg, in the present study. Furthermore, a difference in sedation is unlikely to explain the lack of results for chlorpheniramine because both drugs were noted to reduce activity to a similar extent. This reduced activity observed was not paralleled by the characteristic crouched posture and extensive piloerection that could indicate heightened stress or arousal rather than calming effect (Hennessy et al., 2001). Therefore, the results obtained after hydroxyzine treatment can be interpreted with confidence in terms of anxiolytic effect. One plausible explanation is that the effect of hydroxyzine is not mediated solely via its blockade of histamine H1 receptors. Hydroxyzine has been reported to be about as potent in its binding to 5-HT2 receptors as it is to H1 receptors (Snowman and Snyder, 1990). Because 5-HT2 receptor blocking agents have been reported to possess tranquilizing and anxiolytic activity (Blackburn, 1991; Bersani et al., 1991; Graeff et al., 1998), it could be speculated that the combined blockade of H1 and 5-HT2 receptors might result in a significant decrease in vocalizations. Although effects on separation-induced vocalization have been investigated for a number of 5-HT acting drugs, including those acting on 5-HT1A and 5-HT1D receptors (see Miczek et al., 1995 for review; Molewijk et al., 1996); a possible modulatory role of 5-HT2 receptors in this paradigm does not appear to have been considered. Further studies are therefore necessary to evaluate this hypothesis.

Hydroxyzine has been widely used in humans since the early 1960s. During the last decade, several well-controlled clinical studies have been performed which establish its antianxiety potential, particularly in generalised anxiety disorders (Argyropoulos et al., 2000; Bandelow et al., 2002; Khalid-Khan et al., 2002). Despite its long clinical history, there are very few preclinical publications which relate to anxiolytic-like activity of hydroxyzine in animal models sensitive to anxiolytic or antidepressant drugs (Olson and Whittaker, 1963; Porsolt et al., 1989) The present study is therefore important because it indicates that hydroxyzine is effective in a paradigm sensitive to drugs which are used to treat anxio-depressive states in humans.

Histamine H3 receptors were originally discovered as presynaptic autoreceptors regulating the release of histamine (Arrang et al., 1983). They were subsequently found to also act as heteroreceptors involved in the presynaptic regulation of the release of several neurotransmitters important in the control of arousal (Leurs et al., 1998). Moreover, there is evidence that H3 presynaptic receptors are involved in the regulation of pituitary hormones released during stress (Soe-Jensen et al., 1993; Vohora et al., 2001). Immepip, a potent and brain-penetrating selective H3 agonist, has been shown to decrease the synthesis and the release of brain histamine in vitro and in vivo (Vollinga et al., 1994; Jansen et al., 1998). Thus, theoretically, immepip, administered systemically at the doses tested, would be expected to oppose brain histamine release and HPA axis activation during stressful conditions and consequently, to decrease behavioral responses to stress. The finding that immepip was inactive in the present study does not support such a hypothesis. However, our results are in accordance with those of Perez-Garcia et al. (1999), who showed that R-alpha-methylhistamine, another selective H3 agonist, was devoid of any effect at 10 mg/kg in rats tested in an elevated plus-maze test of anxiety or in the forced swimming test for antidepressant screening. At the dose tested, R-alpha-methylhistamine would be expected to penetrate into the brain (Soe-Jensen et al., 1993). Another factor which might account for the lack of effect of immepip in the present paradigm is the choice of species because the studies showing that H3 agonists modulate brain histamine and stress-induced HPA axis activation were performed in the rat (Soe-Jensen et al., 1993; Jansen et al., 1998; Westerink et al., 2002). However, an explanation in terms of species difference is unlikely because several studies have indicated similarities between rats and guinea pigs in terms of homology, CNS distribution and binding affinities of H3 agonists (Tardivel-Lacombe et al., 2000; Ireland-Denny et al., 2001). Clearly, there is a paucity of published results on the psychopharmacological effects of H3 agonists and further studies using different tests and species sensitive to psychoactive drugs are required to shed light on the behavioral consequences of H3 receptor activation.

The present results demonstrated that our conditions were sensitive to the reference antidepressants, fluoxetine and imipramine, as well as the prototypical benzodiazepine, chlordiazepoxide, all of which decreased transient maternal-separation-induced distress vocalization in the guinea pig. These results confirm earlier published findings using the same paradigm and species (Rupniak et al., 2000; Steinberg et al., 2001). However, chlordiazepoxide at 10 mg/kg inhibited vocalization by only 50% which, albeit statistically significant, contrasts with the total inhibition of vocalization obtained by Rupniak et al. (2000) following chlordiazepoxide at this dose. Apart from a difference in route of administration (intraperitoneal versus subcutaneous), there is no immediate explanation for this discrepancy. Interestingly, in the present study, it was observed that chlordiazepoxide markedly reduced the intensity of vocalization although this was not quantified. It is possible that a certain level of intensity of vocalization may have been used as a criterion for vocalization by Rupniak et al. (2000), although, like the present study, Rupniak et al. recorded duration of audible sound. In contrast, the method used for guinea pig pups by Molewijk et al. (1996) involved the use of sonographic recordings to define the vocalization criterion which then allowed counting of individual calls. Like Rupniak et al. (2000), Molewijk et al. (1996) reported dose-dependent inhibition by benzodiazepines, and clearly, their method would have filtered out the low intensity muffled vocalization which we detected in benzodiazepine-

treated animals. Other than the studies by Molewijk et al. (1996) and Rupniak et al. (2000), there are no detailed published results on the effect of benzodiazepines in the guinea pig pup separation test. More recent publications used this paradigm as an animal model of stress and depression and therefore did not include benzodiazepines as positive controls (Hennessy et al., 2001; Steinberg et al., 2001; Griebel et al., 2002). Interestingly, a review by Panksepp (1998) refers to marked species variability with respect to ability of benzodiazepines to inhibit distress vocalization and notes that benzodiazepines are less effective in inhibiting separation-induced calls than in inhibiting fear. In contrast to the benzodiazepines, the replicated marked effects of tricyclic or 5-HT uptake inhibiting antidepressant drugs is not disputed.

This distinction is pertinent to the interpretation of this paradigm as an animal model. Borsini et al. (2002) reported in a review paper that the isolation-induced vocalization in guinea pig pups was the only paradigm revealing an anxiolytic-like effect of antidepressant drugs after acute administration. The authors therefore proposed this test as the most appropriate to predict activity compatible with drugs currently used to treat anxiety disorders, i.e., antidepressant drugs. Alternatively, several lines of behavioral and anatomo-functional evidence support the notion that isolation-induced vocalization in guinea pig pups might be a model of panic attack (see a review in Panksepp, 1998). In this context, a publication by Kyuhou and Gemba (1998) showed that the electrical stimulation of the midbrain rostral periaqueductal gray (PAG) in guinea pigs induced vocalization which resembles the typical distress calling produced by maternal separation in guinea pig pups. In view of the recognized importance of this brain area in the control of autonomic and neurobehavioral arousal that subserves panic attacks (Jenck et al., 1995), there appears to be reasonable construct validity to support this paradigm as a model of panic attack. However, in that case, it remains to be explained why fluoxetine is very effective in this test, although this drug is not reported to be effective against panic attack. Clearly, further basic studies, including refinement of the method to define the criterion of what constitutes vocalization, as well as extending the pharmacology to include other drug classes, are necessary to elucidate the validity of this model for affective states.

In conclusion, the present data indicate that the antihistamine hydroxyzine is effective in suppressing maternal-separation-induced vocalization in guinea pigs. Such an effect is consistent with known anxiolytic effects in man and provides important animal data for future reference. A possible therapeutic application to panic attack could also be indicated. However, it is likely that nonhistaminergic effects are involved in the action of hydroxyzine in the guinea pig pup test because neither the selective H1 receptor antagonist, chlorpheniramine, nor the selective H3 receptor agonist, immepip, was able to significantly decrease the distress calls. This challenges the concept that all brain-penetrating histamine H1 receptor antagonists will be equivalent in producing tranquilizing effect and point to a minor role for brain histamine systems in the modulation of isolation-induced vocalization in guinea pigs. Further behavioral and pharmacological studies are necessary to better understand this model and its validity for detecting drugs having a potential use in affective disorders.

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