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Short communication

Systemic EMD 68843 injections reduce anxiety in the shock-probe, but not the plus-maze test

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Abstract

Selective serotonin (5-hydroxytryptamine; 5-HT) reuptake inhibitors and 5-HT_{1A} receptor agonists are believed to reduce anxiety. In the present study we examined the effects of injections of 5-{4-[4-(5-cyano-3-indolyl)-butyl]-1-piperazinyl}-benzofuran-2-carboxamide hydrochloride salt (EMD 68843), a 5-HT_{1A} receptor agonist and selective 5-HT reuptake inhibitor, in two animal models of anxiety, plus-maze and shock-probe. Rats received intraperitoneal injections of vehicle, diazepam (2.5 mg/kg), or EMD 68843 (10, 20, or 40 mg/kg) 1 h prior to testing. Diazepam at the single dose tested and EMD 68843 dose-dependently (significantly at 20 and 40 mg/kg) reduced burying in shock-probe. However, only diazepam significantly increased open arm exploration in the plus-maze. Therefore, EMD 68843 has task specific anxiolytic properties.

Keywords: EMD 68843; 5-HT (5-hydroxytryptamine, serotonin) reuptake inhibitor, selective; 5-HT_{1A} receptor agonist; Anxiety

1. Introduction

Selective serotonin (5-hydroxytryptamine; 5-HT) reuptake inhibitors are believed to reduce fear in some animal models of anxiety (Handley, 1995). Selective 5-HT reuptake inhibitors increase extracellular 5-HT levels and decrease the firing of the raphe nucleus by stimulating 5-HT_{1A} autoreceptors (Adell and Artigas, 1991). But the selective 5-HT reuptake inhibitor induced increase in 5-HT is restricted by the negative feedback from 5-HT autoreceptors reducing 5-HT release (Hjorth and Auerbach, 1994; Hjorth, 1996). As a result, desensitization of the 5-HT autoreceptors by chronic selective 5-HT reuptake inhibitor administration (Blier et al., 1990; Goodwin et al., 1985; Kreiss and Lucki, 1995) might be required to normalize 5-HT release before the clinical properties of selective 5-HT reuptake inhibitors are observed (Albert et al., 1996).

The drug 5-{4-[4-(5-cyano-3-indolyl)-butyl]-1-piperazinyl}-benzofuran-2-carboxamide hydrochloride salt (EMD 68843) is a selective 5-HT reuptake inhibitor, which also stimulates presynaptic 5-HT_{1A} autoreceptors (Bartoszyk et al., 1996). For the 5-HT_{1A} receptor agonists their anxiolytic effects have been demonstrated to be mediated directly by activation of 5-HT_{1A} autoreceptors in the raphe nucleus (e.g., Jolas et al., 1995). Long term administration of 5-HT_{1A} receptor agonists desensitizes the 5-HT_{1A} receptor (Jackson et al., 1994; Larsson et al., 1990), but even a single administration of a 5-HT_{1A} receptor agonist can desensitize the 5-HT_{1A} autoreceptors (O'Connell and Curzon, 1996; Rényi et al., 1992). Thus, EMD 68843 might reduce anxiety acutely. To date the effects of EMD 68843 on anxiety have only been shown in the ultrasonic vocalization test (Bartoszyk et al., 1997), an animal model for anxiolytic drug action (De Vry et al., 1993). In the present study we examined the effects of EMD 68843 on two other well known measures of anxiety; the elevated plus-maze and the shock-probe burying tests (Pellow et al., 1985; Treit, 1990).

2. Materials and methods

2.1. Subjects

The subjects were naïve, male albino Sprague–Dawley rats, purchased from Ellerslie (Canada), weighing 300–400 g at the time of testing. Each rat was individually housed in a polycarbonate cage and maintained on a 12:12 h light/dark cycle (lights on at 0700 h), with food and water

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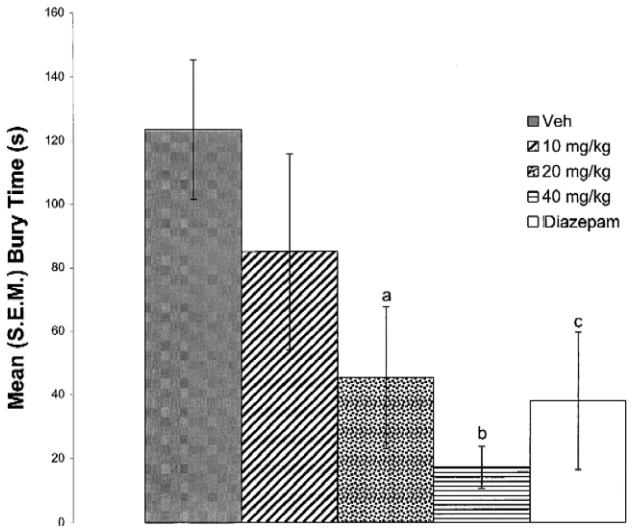

Fig. 1. Mean (±S.E.M.) bury time (s) in the shock-probe apparatus after systemic injections of EMD 68843 (10, 20, or 40 mg/kg), diazepam (2.5 mg/kg), or vehicle (one drop Tween 80 suspended in 10 ml 0.9% saline). [a]$P = 0.02$, [b]$P = 0.001$, and [c]$P = 0.01$ compared with the vehicle control group.

available ad libitum. Behavioral testing occurred between 0900 and 1700 h. Ethical approval for the experiment was obtained and the treatment of all animals complies with the guidelines for the use of experimental animals.

2.2. Injection procedures

The tame, hand-held rats were acclimated to the room in which they were injected for at least 1 h prior to systemic injection. Rats were randomly assigned to one of the following drug conditions for shock-probe testing: a control condition, injected with vehicle (1 drop of Tween 80 in 10 ml 0.9% saline), a positive-control condition, injected with 2.5 mg/kg diazepam (Hoffman, La Roche Limited), and the EMD condition, injected with ultrasonicated 10, 20, or 40 mg/kg EMD 68843 (batch EE 77485, Merck, Darmstadt, Germany). The doses of EMD 68843 were selected based on pilot studies in our laboratory. Drugs were given intraperitoneally (application volume 1 ml/kg bodyweight). Drug conditions were counterbalanced for subsequent plus-maze testing.

2.3. Behavioral testing

The behavioral testing procedures were the same as those used in previous experiments (Treit, 1990; Treit et al., 1993). All behavior was recorded on videotape for ensuing analysis. Shock-probe testing occurred following four consecutive days of handling (3 min each per day) and acclimation to the shock-probe apparatus (30 min each per day). The same rats were then tested in plus-maze following an additional three consecutive days of handling (3 min each per day). All behavioral testing occurred 1 h following the systemic injections. This time interval was chosen based on pilot work in our laboratory.

2.4. Statistical analysis

Results are expressed as means and standard errors of the mean (S.E.M.). The shock-probe and plus-maze data were analyzed using analysis of variance (ANOVA) and post hoc comparisons were made using Fischer's adjusted least significant differences tests (LSD). The statistical significance criterion was defined with an alpha level of 0.05.

3. Results

3.1. Shock-probe

Fig. 1 indicates that injections of EMD 68843 or diazepam produced anxiolytic-like effects in the shock-probe burying test [$F(4,52) = 3.57$, $P = 0.01$]. Rats infused with

Table 1
Mean (±S.E.M.) activity and reactivity in the shock-probe burying task and open arm exploration in the elevated plus-maze

	Shock-probe				
	Vehicle ($n = 11$)	EMD 68843 10 mg/kg ($n = 11$)	EMD 68843 20 mg/kg ($n = 12$)	EMD 68843 40 mg/kg ($n = 11$)	Diazepam 2.5 mg/kg ($n = 12$)
Immobility (s)	22.5 (12.4)	50.9 (31.9)	15.7 (6.58)	35.1 (14.3)	147.9 (92.0)
Shock reactivity	2.32 (0.15)	1.89 (0.21)	2.13 (0.13)	2.05 (0.11)	1.97 (0.07)
Shock number	1.64 (0.24)	1.73 (0.30)	1.50 (0.15)	1.55 (0.16)	2.25 (0.30)
	Plus-maze				
	($n = 11$)	($n = 13$)	($n = 11$)	($n = 13$)	($n = 9$)
% Open arm entries	21.6 (5.16)	17.2 (4.51)	10.2 (5.04)	30.4 (9.80)	46.1[a] (4.65)
% Open arm time	19.3 (6.42)	14.0 (4.42)	8.9 (5.39)	29.1 (10.6)	44.8[b] (7.32)
Total arm entries	9.6 (1.05)	8.9 (0.89)	10.3 (0.75)	9.5 (0.75)	12.6 (1.32)
Closed arm entries	7.5 (0.89)	7.2 (0.75)	9.2 (0.83)	6.9 (1.24)	6.4 (0.53)

[a]$P = 0.01$ vs. vehicle.
[b]$P = 0.02$ vs. vehicle.

20 mg/kg of EMD 68843 ($P = 0.02$), 40 mg/kg of EMD 68843 ($P = 0.001$), or 2.5 mg/kg of diazepam ($P = 0.01$) displayed significantly lower burying levels than their vehicle infused controls. This anxiolytic-like effect occurred in the absence of any significant changes in immobility [$F(4,52) = 1.41$, $P = 0.24$], number of shocks received [$F(4,52) = 1.63$, $P = 0.18$], or shock reactivity [$F(4,52) = 1.38$, $P = 0.25$, see Table 1]. Injections of 10 mg/kg of EMD 68843 did not affect burying activity.

3.2. Plus-maze

The table indicates that injections of EMD 68843 did not affect open arm activity in the plus-maze at the doses used, whereas injections of diazepam did have a significant effect on both percent open arm time [$F(4,52) = 3.26$, $P = 0.02$] and percent open arm entries [$F(4,52) = 3.93$, $P = 0.01$]. There was no indication of non-specific changes in general activity, as neither the total number of arms entered [$F(4,52) = 1.97$, $P = 0.11$] nor the number of closed arms entered [$F(4,52) = 1.18$, $P = 0.33$, see Table 1] differed significantly between groups.

4. Discussion

In the present experiment, we determined that EMD 68843 is anxiolytic in the shock-probe test, but not in the elevated plus-maze test. In addition, the anxiolytic effects of EMD 68843 occur over a specified dose range. The reduction in anxiety observed in the shock-probe apparatus following systemic EMD 68843 injections was similar to that seen after the systemic administration of diazepam, a well-characterized anxiolytic compound. All rats avoided the shock-probe to a similar extent, suggesting they could associate the shock with the probe. Moreover, the activity and reactivity levels for all drug conditions were similar to those obtained for systemic vehicle injections. This indicates that the effects on anxiety were not confounded by an influence on other variables.

Our results are consistent with other studies, which indicate that the acute administration of EMD 68843 reduces anxiety (Bartoszyk et al., 1997). Traditionally it is believed that a chronic administration of selective 5-HT reuptake inhibitors is required before clinical effects can be observed. Since EMD 68843 is a presynaptic 5-HT_{1A} receptor agonist as well as a selective 5-HT reuptake inhibitor, our results support the notion that acute effects of selective 5-HT reuptake inhibitors are possible provided that pre-synaptic 5-HT_{1A} autoreceptors are desensitized. An interesting follow-up of the present study would be to compare the acute with the chronic administration of EMD 68843. If EMD 68843 produces its acute effects through a desensitization of pre-synaptic 5-HT_{1A} autoreceptors, then one might expect to see an increase in its anxiolytic effects after chronic administration. But, thus far the acute effects of EMD 68843 are rather explained by its direct stimulation of 5-HT_{1A} autoreceptors; in the ultrasonic vocalization test, the anxiolytic effect of EMD 68843 was blocked by the potent 5-HT_{1A} receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinyl-cyclohexane carboxamide maleate (WAY 100635; Bartoszyk et al., 1997).

It is not surprising that we failed to obtain anxiolytic effects in the elevated plus-maze after acute systemic EMD 68843 injections. In previous studies of serotonergic drugs, the elevated plus-maze has given rise to the greatest variability. For example, chronic systemic injections of the selective 5-HT reuptake inhibitor fluoxetine failed to affect anxiety measures in the elevated plus-maze in one study (Durand et al., 1999), while acute administration of the selective 5-HT reuptake inhibitor citalopram increased anxiety in the elevated plus-maze in other studies (Matto et al., 1996; Pollier et al., 2000). For 5-HT_{1A} receptor agonists such as 8-hydroxy-2-(n-dipropylamino) tetraline hydrobromide (8-OH-DPAT), besides some anxiolytic effects, most studies reported lack of anxiolytic or even anxiogenic effects (Griebel, 1995). The absence of an effect on anxiety in the elevated plus-maze in our study is unlikely to have resulted from an incorrect dose or time interval. Both variables were extensively explored in pilot work. Again, to compare the acute with the chronic administration would be interesting.

In summary, acute EMD 68843 is anxiolytic in shock-probe, but not the elevated plus-maze, over a specific dose range. This anxiolytic effect is comparable to that obtained with the systemic administration of diazepam, a well-characterized anxiolytic compound.

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