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Inhibition of shock-induced foot tapping behaviour in the gerbil by a tachykinin NK_1 receptor antagonist

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Abstract

The selective tachykinin NK_1 receptor antagonist, 2-(*R*)-(1-(*R*)-3,5-Bis(trifluoromethyl)phenylethoxy)-3-(*S*)-(4-fluoro)phenyl-4-(3-oxo-1,2,4-triazol-5-yl)methylmorpholine (MK-869), has been recently described as a novel therapeutic approach for anxiety/depression. A frequently used model to establish the central nervous system (CNS) activity of tachykinin NK_1 receptor antagonists is the inhibition of NK_1 agonist-induced foot tapping in gerbils. In the present study, we demonstrate that foot tapping can also be induced in most, but not all, gerbils by footshock and associated cues. MK-869 (0.3–3 mg/kg, i.p.) dose-dependently blocked this foot tapping response. This effect was further shown to be due to selective NK_1 receptor blockade, since (2*S*,3*S*)-*cis*-3(2-methoxybenzylamino)-2-phenylpiperidine (CP-99,994; 3 mg/kg, i.p.) inhibited foot tapping, whereas its less active enantiomer (2*R*,3*R*)-*cis*-3(2-methoxybenzylamino)-2-phenylpiperidine (CP-100,263; 3 mg/kg, i.p.) had no effect. Diazepam (1–10 mg/kg, i.p.) also inhibited foot tapping, whereas fluoxetine (10–30 mg/kg, i.p.) markedly increased this behaviour. The present data support the view that foot tapping in the gerbil is a behavioural response to an aversive stimulus, and is robustly inhibited by two NK_1 receptor antagonists. The data support a role for tachykinin NK_1 receptor antagonists as novel anxiolytic/antidepressants.

Keywords: Tachykinin NK_1 receptor antagonist; Foot tapping; (Gerbil); Fear conditioning; Anxiety; Depression

1. Introduction

Following the recent publication of Kramer et al. (1998), there has been considerable interest in Neurokinin (NK)1 receptor antagonists as novel treatments for anxiety and depression. These workers reported that in a 6-week double-blind, placebo-controlled study in patients with major depression, the selective NK_1 receptor antagonist, 2-(*R*)-(1-(*R*)-3,5-Bis(trifluoromethyl)phenylethoxy)-3-(*S*)-(4-fluoro)phenyl-4-(3-oxo-1,2,4-triazol-5-yl)methylmorpholine (MK-869) (300 mg/day), produced a positive outcome as measured by both the Hamilton depression (HAM-D21) and anxiety (HAM-A) scales. The effects were equivalent to that of the selective serotonergic reuptake inhibitor, paroxetine (20 mg/day), and side effects typically associated with this drug class, e.g. nausea, sexual dysfunction were diminished in the MK-869 group. Taken together, the study of Kramer et al. (1998) may represent an important advance in the search for novel treatments of anxiety and depression (Nutt, 1998).

In gerbils, a species whose NK_1 receptor pharmacology resembles that of the human (Gitter et al., 1991; Beresford et al., 1991), the intracerebroventricular (i.c.v.) administration of the NK_1 agonist, GR73632 (D-Ala [L-Pro9, Met-Leu10]-substance P-(7–11)), produces a characteristic rhythmic tapping of the hind feet (Graham et al., 1993). This robust and readily quantifiable response is inhibited by brain-penetrating antagonists of the NK_1 receptor (Graham et al., 1993; Rupniak and Williams, 1994; Bristow and Young, 1994). Consequently, NK_1 agonist-induced foot tapping in the gerbil has become a valuable in vivo assay for the identification of centrally acting tachykinin NK_1 receptor antagonists (Rupniak et al., 1997; Hale et al., 1998). Since foot tapping in the gerbil has also been reported following electroshock or offset of reward it is postulated to be a species specific response to an aversive stimulus (Routtenberg and Kramis, 1967). Given

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the clinical evidence that NK_1 receptor antagonists may have antidepressant/anxiolytic properties (Kramer et al., 1998), and the robust nature of foot tapping behaviour following NK_1 agonist administration, we sought to investigate ways of non-pharmacologically inducing this behaviour in gerbils.

In the present study, we have used a Pavlovian fear-conditioning procedure. In a variety of species, the pairing of specific cues (conditioned stimulus) with an aversive stimulus (unconditioned stimulus), typically electroshock, subsequently results in the conditioned stimulus inducing a variety of fear related behaviours (see LeDoux, 1998; Davis, 1999 for reviews). In rodents, this typically involves freezing or increased startle responses, as well as autonomic signs such as defaecation, increased heart rate and arterial blood pressure (LeDoux et al., 1988; Davis, 1999). Following the establishment of suitable shock parameters in gerbils, we studied their behaviour both during the conditioning session and also in a retest session, performed 24 h later where the animals are presented with the conditioned stimulus in the absence of the unconditioned stimulus. We found that foottapping behaviour could be induced in some, but not all, gerbils during both the conditioning and retest session. Consequently, we studied the effect of the tachykinin NK_1 receptor antagonists MK-869 (Hale et al., 1998) and (2*S*,3*S*)-*cis*-3(2-methoxybenzylamino)-2-phenyl piperidine (CP-99,994) (McLean et al., 1993), as well as a clinically efficacious anxiolytic (diazepam) and antidepressant (fluoxetine) against this shock-induced foot tapping. Some of this work has been published in abstract form (Ballard et al., 1999).

2. Materials and methods

2.1. Subjects

Male and female Mongolian gerbils (Biological Research Laboratories, Füllinsdorf, Switzerland and Charles River, USA), weighing between 40 and 70 g, were used in all experiments. Gerbils were housed four per cage with food and water available ad libitum, in temperature and humidity-controlled holding rooms. The animals were allowed 4–7 days to acclimatize to the housing conditions prior to testing. All testing was conducted during the light phase of the light/dark cycle (lights on: 0600–1800 h). Experimentally naive gerbils were used in each study. All experiments were carried out under the guidelines issued under local Cantonal and Swiss federal law.

2.2. Shock-induced foot tapping

The test apparatus consisted of a Perspex chamber ($14 \times 14 \times 13$ cm [$L \times W \times H$], Med Associates, USA) with a grid floor through which a scrambled electrical stimulus could be applied. A cue light was located on one wall and a sonalert system on the ceiling, which was capable of delivering a 2900-Hz tone. Two visually distinct chambers were used, one of white perspex, the other black perspex. In all other aspects, the chambers were essentially identical. Gerbils were run in the same chamber during both the conditioning and retest session. Also, the chamber type was balanced across all treatment groups. The delivery of electroshock and the presentation of the light and tone conditioned stimulus was controlled by Med PC (Med Associates, USA).

Preliminary experiments suggested that shock levels between 1 and 2 mA were required to induce responses such as flinch, vocalisation and jump in gerbils. At levels below 1 mA, no consistent response to footshock was observed. Consequently, an experimental protocol was designed whereby a 2-min initial familiarisation period was followed by 6×1 s footshocks delivered at 60-s intervals. The onset of each electrical stimulus was preceded by a 30-s light/tone conditioned stimulus. Three shock intensities were tested: 0 mA ($n = 11$); 1 mA ($n = 11$); 2 mA ($n = 13$). The total duration of the conditioning session was 8 min. In a retest session performed 24 h later, the animals were reexposed to the conditioning box for a 3-min period followed by three presentations of the 30-s conditioned stimulus separated by 30-s intervals. Thus, the total duration of the retest session was 6 min. At no time was footshock delivered during this retest session. The time spent on foot tapping and the immobility time (i.e., freezing) were recorded at each 30-s time bin, and the number of faecal boli were counted at the end of each session. Immobility time was operationally defined as total immobility of the animal except for respiratory movement.

In experiments to study the effect of drug treatment on shock-induced foot tapping, gerbils were pretreated prior to placement into the conditioning box where they were subjected to 6×1 s electrical stimuli (2 mA) timed at 60-s intervals. Each footshock was signalled by presentation of a 30-s light/tone cue (conditioned stimulus). MK-869 ($n = 14$–26 per group)-treated gerbils were retested 24 h later (without drug administration). CP-99,994 ($n = 10$–12 per group), diazepam ($n = 9$–10 per group; $n = 4$ at 10 mg/kg) and fluoxetine ($n = 10$–12 per group) were only studied on the conditioning session. To determine whether drug treatment altered shock perception, the percentage of animals displaying each of the following responses: flinch, vocalisation and jump, at least once during the session was calculated.

During the course of these experiments, a small proportion of gerbils developed seizures on placement into the test chamber. These animals were always excluded from further study. Differences in group sizes usually reflect this adjustment.

2.3. Tachykinin NK_1 agonist-induced foot tapping

Gerbils were anaesthetised by inhalation of isoflurane/oxygen mixture, the scalp was exposed and GR73632

(0.3–10.0 pmol/5 μl) or vehicle (0.1% bovine serum albumin) was administered into the lateral ventricles (i.c.v.) via a cuffed 25-gauge needle vertically inserted to a depth of 4.5 mm below bregma. The incision was closed using a clip suture and gerbils were placed into perspex boxes on recovery of their righting reflex. Duration of foot tapping behaviour over a 5-min period was measured.

2.4. Drugs and injections

MK-869, CP-99,994, CP-100,263 ((2*R*,3*R*)-*cis*-3(2-methoxybenzylamino)-2-phenylpiperidine), GR73632, diazepam and fluoxetine were all synthesised within the Chemistry department at F. Hoffmann-La Roche (Basel). All test substances were dissolved in 0.3% Tween 80 v/v 0.9% NaCl and administered in an injection volume of 10 ml/kg intraperitoneally (i.p.) 30 min prior to testing. GR73632 was dissolved in 0.1% bovine serum albumin in water and frozen (−20°C) in aliquots until use, where it was given by direct intracerebroventricular injection.

2.5. Statistical analysis

One-factor Analysis of Variance (ANOVA) followed, in significant cases, by Fisher's Least Significant Difference test was used to analyse the immobility time and

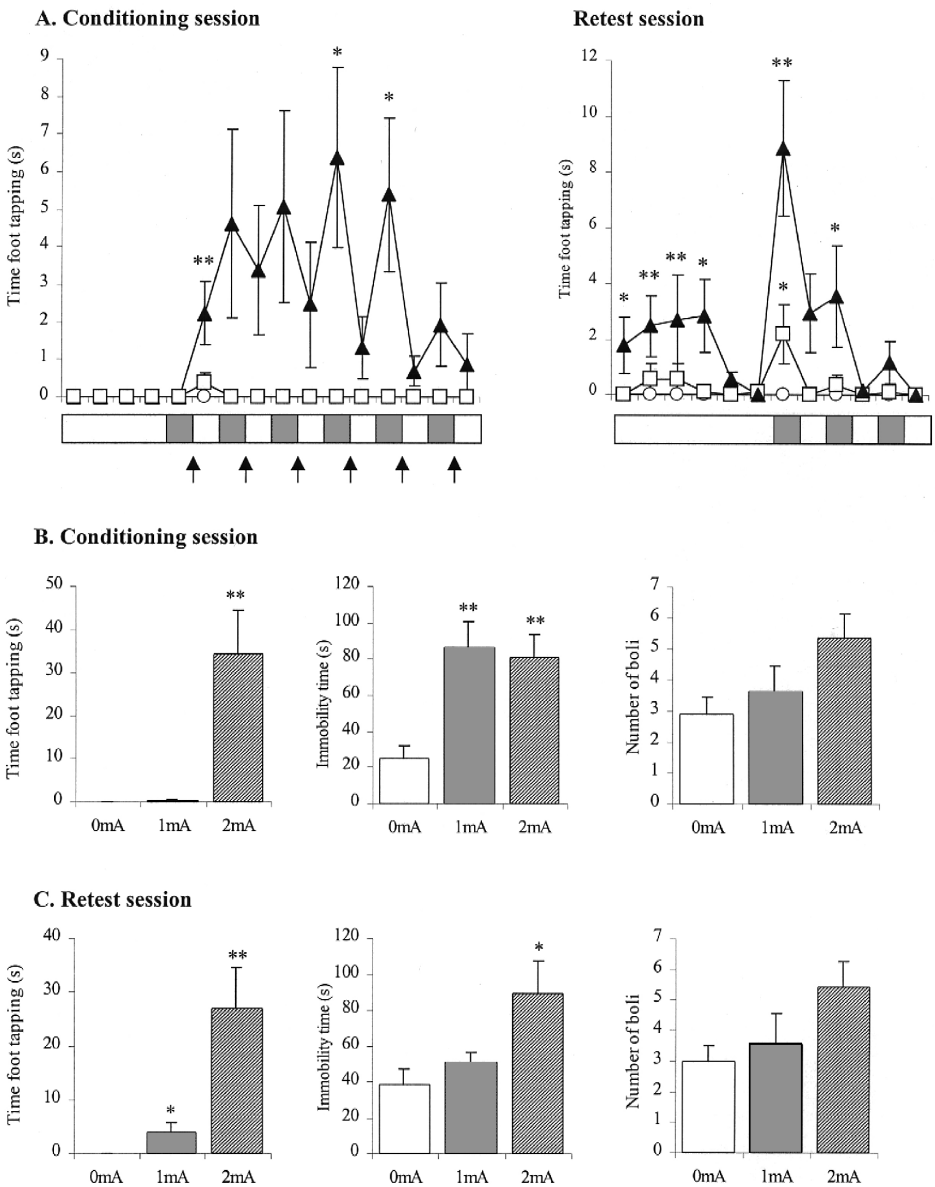

Fig. 1. Characterisation of various behaviors in gerbils during a fear-conditioning procedure and during a retest session performed 24 h later where the gerbils were presented with cues previously paired with footshock presentation (see Materials and methods for further details). (A) Temporal distribution of foot tapping behaviour recorded at 30-s time bins over the conditioning and retest session. ○ = No shock controls, □ = 1 mA intensity, ▲ = 2 mA intensity. The shaded area along the *x*-axis indicates the conditioned stimulus (light + tone) presentation, the vertical arrows indicate shock presentation (unconditioned stimulus). Data collapsed for foot tapping, freezing and defaecation over (B) the conditioning, and (C) the retest session are also shown. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. 0 mA vehicle controls.

defaecation scores. Since foot tapping was not observed in all gerbils, and marked inter-animal differences were found in those that did respond, this measure was analysed by Kruskal–Wallis test followed by post hoc Mann Whitney *U*-test. Correlation coefficients comparing: (1) immobility time and foot tapping scores in the conditioning and retest session; and (2) foot tapping scores in the conditioning and retest sessions, were calculated for shocked controls in the characterisation of shock-induced foot tapping experiment. All statistical analysis was conducted using StatView for Windows (Version 5.0.1, SAS Institute).

3. Results

3.1. Characterisation of shock-induced foot tapping in gerbils

Following placement in the test chambers, over the initial 2-min (unshocked) period, all gerbils showed reasonable activity with minimal freezing and no foot tapping behaviour. However, following the first conditioned stimulus–shock pairing, there was a marked incidence of foot tapping in the 2 mA, but not in the control (no shock) or 1 mA group. Although in percentage terms, the mean amount of time engaged in this behaviour was relatively small (6–20%), it was maintained over successive conditioned stimulus–shock pairings, reaching significance during the fourth and fifth conditioned stimulus presentation (Fig. 1A), but by the sixth conditioned stimulus–shock pairing the incidence of foot tapping was in decline. During the course of this experiment, it was evident that not all gerbils within the 2-mA group demonstrated reliable foot tapping behaviour—the actual proportion being 85%. Inclusion of responders and non-responders yielded an overall score collapsed over the entire 8-min session of 34 ± 10 s (range: 0–97 s; Fig. 1B). Immobility time was indistinguishable between groups over the initial 2 min, although following the first conditioned stimulus–shock pairing its incidence increased in both the 1- and 2-mA groups, which were similar, although both were significantly different to the control (unshocked) group ($F(2,32) = 7.6$, $P < 0.01$) (Fig. 1B). Although a modest increase in defaecation was recorded in the 2-mA group, this narrowly failed to reach significance ($F(2,32) = 3.2$, $P = 0.06$) (Fig. 1B). Comparison between immobility time and foot tapping scores in the 2-mA group yielded a correlation of borderline significance (correlation coefficient -0.56, $P = 0.04$), suggesting a trend toward higher immobility scores in gerbils having lower foot tapping scores.

In the retest session, some foot tapping was evident during the initial 3-min period in the 2-mA group in which no conditioned stimulus presentations were made. This was presumably a response to contextual cues associated with the foot shock (Phillips and LeDoux, 1992). However, the first light/tone conditioned stimulus presentation produced a marked foot tapping response following both 1 and 2 mA footshock, but again not in all gerbils (Fig. 1A). There was a significant correlation (correlation coefficient $+0.82$, $P < 0.01$) between foot tapping scores recorded during the conditioning and retest session (Fig. 2). A main effect on immobility time was also found in the retest experiment ($F(2,32) = 4.1$, $P < 0.05$), although this measure was only increased in the 2-mA group (Fig. 1C). A comparison between immobility time and time spent foot tapping in the 2-mA group again revealed a trend toward higher immobility scores in gerbils having lower foot tapping scores, however this correlation did not reach significance (correlation coefficient -0.51, $P = 0.07$).

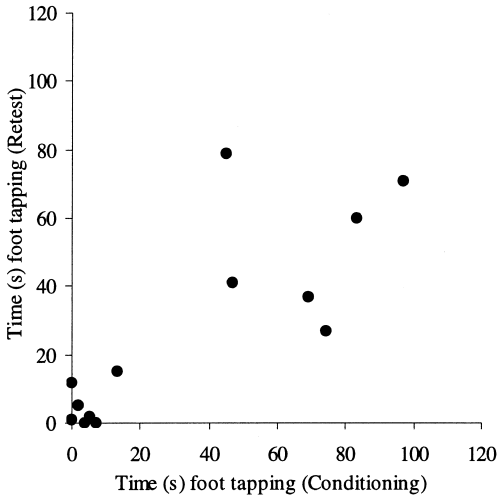

Fig. 2. Correlation between time spent foot tapping in the conditioning session compared to time spent foot tapping in the retest session for gerbils taken from the 2-mA group in experiment 1.

3.2. Effect of the tachykinin NK_1 receptor antagonists MK-869 and CP-99,994 against shock-induced foot tapping

Pretreatment with MK-869 (0.3–3 mg/kg) produced a significant reduction in foot tapping ($H = 23.3$, $DF = 4$, $P < 0.01$) induced by a 2-mA electrical stimulus (Fig. 3A). In this experiment, no overall main effect on the immobility time was found ($F(4,89) = 1.5$, $P = 0.2$), despite the fact that the time engaged in this behaviour appeared to be higher in shocked compared to unshocked controls (Table 1). An overall main effect of defaecation ($F(4,85) = 4.8$, $P < 0.01$) was recorded, due to the difference between shocked and unshocked controls (Table 1). MK-869 did not reduce the shock-induced increase in this measure. MK-869 did not affect shock perception, since all gerbils displayed both vocalisation and flinch response and the majority of animals produced a jump response to the shock (Table 2). In the retest session (Fig. 3B), gerbils that were treated with MK-869 prior to the conditioning session

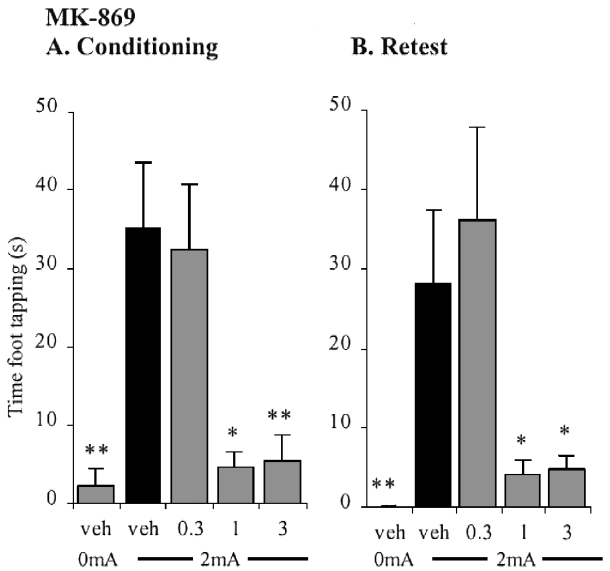

Fig. 3. Effect of MK-869 ($n = 14–26$ per group) on gerbil foot tapping (A) induced by a 2-mA electrical stimulus during the conditioning session and (B) in response to the conditioned stimulus during the retest session. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. 2 mA vehicle controls.

Table 2
Percentage of gerbils displaying flinch, vocalisation or jump in response to footshock

Treatment	Dose (mg/kg)	Flinch	Vocalisation	Jump
MK-869	Vehicle (2 mA)	100	100	69
	0.3	100	100	53
	1	100	100	80
	3	100	100	70
CP-99,994	Vehicle (2 mA)	100	100	50
	3	100	100	46
	CP-100,263	100	100	64
Diazepam	Vehicle (2 mA)	100	100	100
	1	100	100	50
	3	100	100	0
	10	100	100	0
Fluoxetine	Vehicle (2 mA)	100	100	17
	3	100	100	42
	10	100	100	33
	30	90	100	33

produced a significant inhibition of the foot tapping response ($H = 22.9$, $df = 4$, $P < 0.001$). There was no overall main effect on the immobility time ($F(4,89) = 2.3$, $P = 0.07$) (Table 1). MK-869 also did not affect the shock-induced increase in defaecation (Table 1).

CP-99,994 (3 mg/kg) similarly reduced shock-induced foot tapping ($H = 19.7$, $df = 3$, $P < 0.01$), while its less active enantiomer CP-100,263 (3 mg/kg) was inactive

Table 1
Immobility time and number of faecal boli during conditioning and retest sessions

Treatment	Dose (mg/kg)	Conditioning: immobile time (s)	Conditioning: no. of faecal boli	Retest: immobile time (s)	Retest: no. of faecal boli
MK-869	Vehicle (0 mA)	23 ± 5	4 ± 1[a]	30 ± 9	2 ± 1[b]
	Vehicle (2 mA)	42 ± 5	7 ± 1	54 ± 8	6 ± 1
	0.3	42 ± 9	7 ± 1	59 ± 12	7 ± 1
	1	35 ± 4	6 ± 1	30 ± 4	6 ± 1
	3	45 ± 7	7 ± 1	63 ± 12	5 ± 1
CP-99,994	Vehicle (0 mA)	39 ± 11	2 ± 1	NT	NT
	Vehicle (2 mA)	37 ± 7	5 ± 1		
	3	59 ± 15	5 ± 1		
	CP-100,263	59 ± 12	5 ± 1		
Diazepam	Vehicle (0 mA)	28 ± 10	1 ± 1[b]	NT	NT
	Vehicle (2 mA)	45 ± 8	5 ± 1		
	1	55 ± 7	5 ± 1		
	3	205 ± 23[b]	2 ± 1[b]		
	10	261 ± 18[b]	1 ± 1[b]		
Fluoxetine	Vehicle (0 mA)	15 ± 11[c]	5 ± 1	NT	NT
	Vehicle (2 mA)	52 ± 12	7 ± 1		
	3	29 ± 15	6 ± 1		
	10	3 ± 3[a]	6 ± 1		
	30	15 ± 11[c]	4 ± 1		

[a] $P < 0.01$ vs. vehicle (2 mA) group (ANOVA followed by post hoc Fisher's PLSD test).
[b] $P < 0.001$ vs. vehicle (2 mA) group (ANOVA followed by post hoc Fisher's PLSD test).
[c] $P < 0.05$ vs. vehicle (2 mA) group (ANOVA followed by post hoc Fisher's PLSD test).

(Fig. 4A). No overall main effect was found on measures of immobility time ($F(3,39) = 1.0$, $P = 0.4$) and defaecation ($F(3,39) = 2.5$, $P = 0.07$). In this experiment, the control (unshocked) baseline immobility time was relatively high (Table 1). Shock perception was not altered following pretreatment with either CP-99,994 or CP-100,263. All the gerbils tested had a flinch and vocalisation response to the footshock, the jump response was more variable but unrelated to drug pretreatment (Table 2).

3.3. Effect of diazepam and fluoxetine against shock-induced foot tapping

Diazepam (1–10 mg/kg) produced a highly robust inhibition of shock-induced foot tapping ($H = 27.1$, $df = 4$, $P < 0.01$), with the effect at the 1 mg/kg dose being of borderline significance ($P = 0.06$) and complete inhibition at the 3 and 10 mg/kg doses (Fig. 4B). A significant main effect was also found for immobility time ($F(4,38) = 43.0$, $P < 0.01$) and defaecation ($F(4,38) = 9.3$, $P < 0.01$) in this experiment. Although no increase in immobility time was observed for the shocked vs. unshocked group, there was a highly significant increase in this measure in gerbils pretreated with diazepam at the 3 and 10 mg/kg doses, which probably reflects a diazepam-induced reduction in general activity (Table 1). The inhibition of foot tapping was not due to muscle relaxation since diazepam did not significantly affect grip strength (vehicle: 152 ± 23 g ($n = 5$); 3 mg/kg: 130 ± 7 g ($n = 6$);10 mg/kg: 106 ± 14 g ($n = 6$); ANOVA $F(3,19) = 1.6$, $P = 0.2$). Defaecation was significantly reduced relative to shocked controls at these doses. Diazepam treatment did not alter the vocalisation and flinch response to the shock, although at 1 mg/kg the jump response was reduced and at 3–10 mg/kg this response was absent (Table 2).

In contrast to diazepam, fluoxetine (10–30 mg/kg) actually increased the duration of foot tapping relative to shocked controls (Fig. 4C). Indeed, this effect was highly significant, with gerbils recording up to 292 s foot tapping during the test period ($H = 30.0$, $df = 4$, $P < 0.0001$). Fluoxetine also reduced the immobility time ($F(4,50) = 2.9$, $P < 0.05$), this being likely due to the fact that the gerbils under fluoxetine appeared to engage in more active behaviours such as foot tapping. In this experiment, there was no main effect of defaecation ($F(4,50) = 1.3$, $P = 0.3$). Shock perception was unaltered by fluoxetine, since the flinch, vocalisation and jump response did not differ from the vehicle group (Table 2).

3.4. Effect of test compounds against tachykinin NK_1 receptor agonist-induced foot tapping

Intracerebroventricular injection of the NK_1 agonist, GR73632, produced a dose-related incidence of foot tapping throughout the 5-min test session. Indeed at the 3 and 10 pmol doses, the gerbils foot tapped virtually throughout the entire observation period (Fig. 5A), having cumulative scores of 275 ± 8 and 284 ± 4 s at the 3 and 10 pmol doses, respectively. This behaviour was dose-dependently blocked by pretreatment with the tachykinin NK_1 receptor antagonists MK-869 (0.3–3 mg/kg; Fig. 5B) and CP-99,994 (3–10 mg/kg), but not by CP-100,263 (3–10

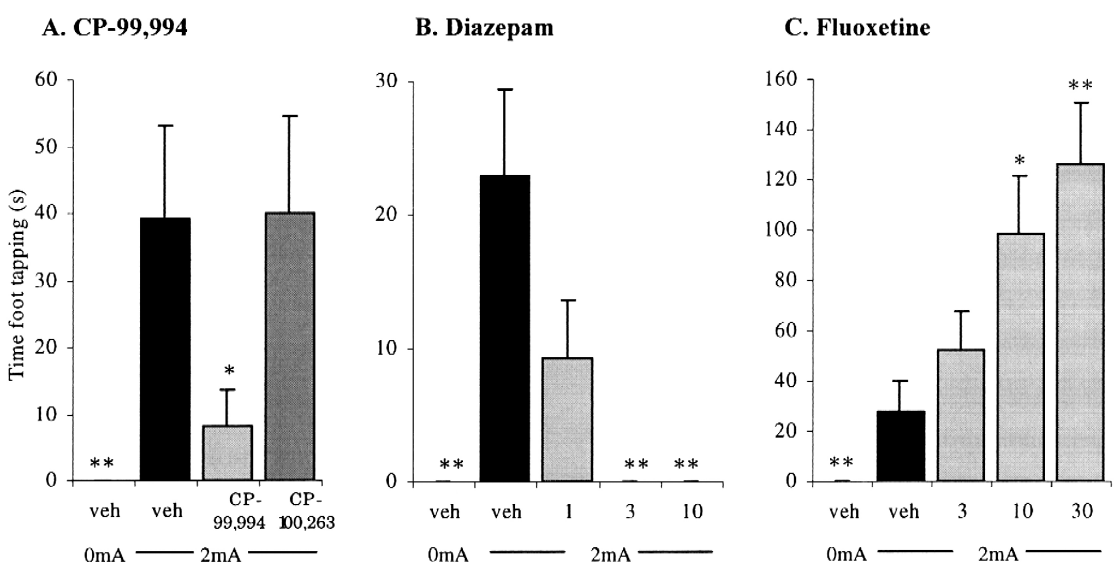

Fig. 4. Effect of (A) CP-99,994 and CP-100,263 ($n = 10$–12 per group); (B) diazepam ($n = 9$–10 per group; $n = 4$ at 10 mg/kg); (C) fluoxetine ($n = 10$–12 per group) on gerbil foot tapping induced by a 2-mA electrical stimulus. Note the different y-axis scales. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. 2 mA vehicle controls.

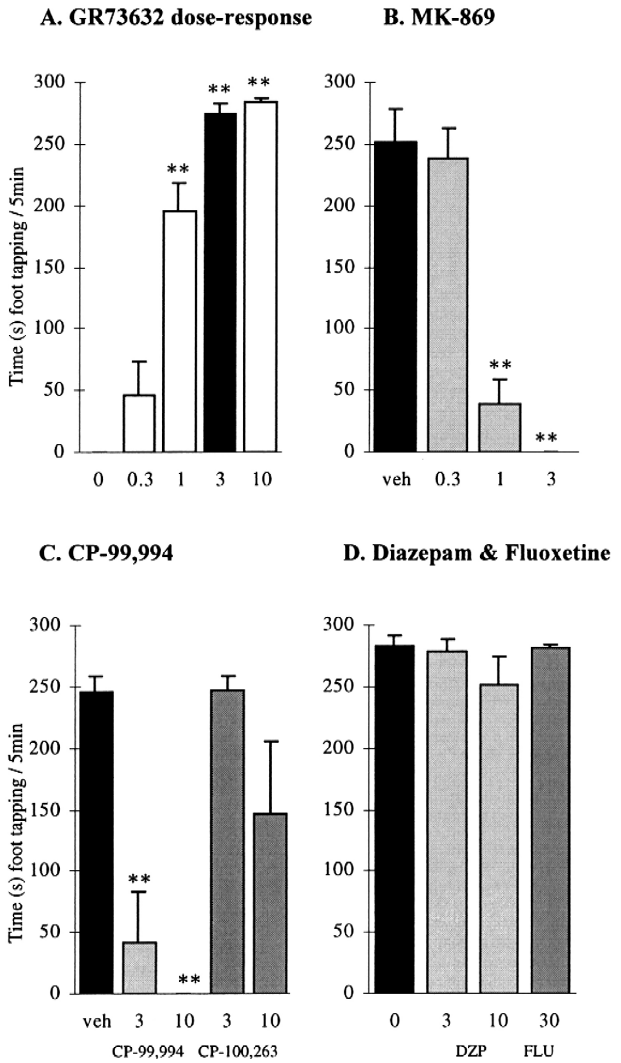

Fig. 5. (A) Dose response for GR73632-induced foot tapping in gerbils. The 3 pmol/5 μl i.c.v. dose was subsequently chosen for antagonist studies. Effect of the tachykinin NK_1 receptor antagonists (B) MK-869, and (C) CP-99,994 against GR73632-induced foot tapping. Note that the less active isomer CP-100,263 was only weakly active in this test. (D) Effect of diazepam (3 and 10 mg/kg) and fluoxetine (30 mg/kg) against GR73632-induced foot tapping ($n = 5–9$ per group). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle pretreated controls.

mg/kg) (Fig. 5C). Neither diazepam (3–10 mg/kg) nor fluoxetine (30 mg/kg) pretreatment affected the foot tapping response produced by GR73632 (Fig. 5D).

4. Discussion

In the present study, we have demonstrated that foot tapping may be elicited in gerbils by an aversive stimulus, i.e. electroshock, and notably by cues paired with this unconditioned stimulus. Such fear-conditioning procedures have been used to induce fear in rodents (LeDoux, 1998; Davis, 1999), the present data suggests foot tapping may be an expression of fear and/or anxiety in the gerbil. Evidence for a neurokinin involvement in this behaviour is supported by the finding that the tachykinin NK_1 receptor antagonists MK869 and CP-99,994 completely block shock-induced foot tapping. Measurement of responsivity to footshock during conditioning, suggested that the effect of both tachykinin NK_1 receptor antagonists was not due to changes in shock perception.

There is clinical evidence that tachykinin NK_1 receptor antagonists produce anxiolytic effects (Kramer et al., 1998), however in preclinical studies this evidence has been limited by the prevalence of tests utilising rats and mice, whose NK_1 receptor pharmacology differs to that of the human receptor (Gitter et al., 1991; Beresford et al., 1991). Nonetheless, the tachykinin NK_1 receptor antagonist (±)-CP-96,345, has been reported to increase the time spent in the more aversive light compartment in a mouse light/dark test, albeit at sedative doses (Zernig et al., 1992). File (1997) also reported anxiolytic activity of the tachykinin NK_1 receptor antagonist, CGP 49823 in a rat social interaction test. Furthermore, NK_1 receptor activation has been shown to induce anxiogenesis in mice, an effect which was inhibited by antagonists at this receptor (Texeira et al., 1996). However, in order to characterise these compounds effectively in animal models of anxiety and depression, it is preferable to develop tests using animal species with a similar NK_1 receptor pharmacology to human, such as gerbils and guinea pigs.

It was for this reason that we examined the present gerbil fear conditioning test. In the initial behavioural characterisation, we also identified increased immobility time (i.e. freezing behaviour) and defaecation during both the conditioning and retest sessions. Indeed immobility time appeared to be the most sensitive measure, since it was significantly increased in the 1-mA group, whilst foot tapping was only evident in the 2-mA group. However, in subsequent experiments, shock-induced changes in this measure became variable, due perhaps to baseline differences seen across studies. Since we attempted no discrimination between immobility and freezing, it was operationally defined as complete cessation of movement except respiratory (e.g., see Phillips and LeDoux, 1992), a more detailed ethological approach to scoring this and other fear related behaviours may be appropriate (e.g., horizontal tail shaking, eye lid closure). Similarly defaecation in subsequent tests gave inconsistent results. Thus, foot tapping emerged as the most robust response—it was rarely seen in unshocked controls (total 2%), and the incidence was reasonably consistent across experiments.

However, in contrast to the NK_1 agonist-induced foot tapping, only 80–85% of the gerbils actually did foot tap following electroshock, and to variable degrees. Hendrie and Starkey (1998) have reported that only male gerbils displayed foot-tapping behaviour in a social interaction test. Yet in the present study, the animals sex did not seem to contribute to the variance. The robustness of this response might be improved by pre-selecting gerbils based

on their foot tap response to another noxious environmental stimulus, however as yet we have not explored this approach. In addition, it is possible that housing conditions may alter this behavioural response (see Clark and Galef, 1979; Hendrie and Starkey, 1998). The reasonable correlation between individual foot tapping scores recorded in the conditioning and test sessions, certainly suggest that individual gerbils differ in their propensity to demonstrate this behaviour. This positive correlation has also been replicated in subsequent experiments. In addition, the comparison between freezing and foot tapping scores in the 2-mA group indicated a trend toward higher freezing/immobility scores in gerbils having lower foot tapping scores. This suggests that these are mutually exclusive behaviours for the expression of fear and/or anxiety, i.e. if an animal is engaged in an 'active' behaviour such as foot tapping, then it is unable to express a 'passive' behaviour such as freezing. Foot tapping has also been shown to occur in other situations, e.g. mating, indicating that this behaviour may not only be an expression of fear, but also of heightened arousal. However, within the present fear conditioning procedure gerbils foot tapped in response to the various cues predictive of footshock, as well as to the footshock itself. This would imply that in the present experimental paradigm, foot tapping is a behavioural response to an aversive stimulus, and a likely index of fear and/or anxiety.

Examination of the temporal distribution of foot tapping during the conditioning session, revealed that this behaviour was more evident during the conditioned stimulus presentation, compared to the intervening time period. Interestingly, rather than a gain in intensity with repeated shock-conditioned stimulus pairings, by the end of the session it was in decline. Whether this reflects a behavioural adaptation or perhaps receptor desensitization (NK_1 receptor internalisation?) is unclear, and may be worthy of further study. Indeed, Smith et al. (1999) reported immunocytochemical evidence for NK_1 receptor endocytosis within the basolateral amygdala following immobilisation stress in gerbils. Since the amygdala is a critical neuroanatomical locus for the formation and storage of information processes relating to mammalian fear conditioning (see LeDoux, 1998; Davis, 1999; Maren, 1999 for recent reviews), one might predict similar changes in this procedure. Indeed, stress-, including shock-induced changes in substance P content have been reported in diverse regions of the central nervous system (CNS) (Bannon et al., 1986; Brodin et al., 1994; Hahn and Bannon, 1999). Furthermore, there is evidence for a substance P projection pathway from the medial amygdaloid nucleus to the medial hypothalamus, which seems to be involved in the expression of defensive rage behaviour (Shaik et al., 1993).

The selective, brain penetrant NK_1 receptor antagonists MK-869 and CP-99,994, but not its less active enantiomer CP-100,263 (McLean et al., 1993; Tattersall et al., 1993), blocked shock-induced foot tapping. This effect occurred at doses that produced no signs of ataxia or myorelaxation. The doses that blocked shock-induced foot tapping were similar to those that blocked the pharmacologically mediated response. Taken together, these data strongly support a role for NK_1 receptors in the mediation of shock-induced foot tapping. Pharmacological dissociations between the inhibition of shock and NK_1 agonist-induced foot tapping were seen with diazepam, which failed to reduce the latter response. This finding essentially eliminates a sedative action of diazepam to account for the antagonism seen in the shock-induced model, as the gerbils treated at these doses of benzodiazepine were clearly capable of emitting this behaviour. Also muscle relaxation was not evident at these doses, since grip strength measures were similar between diazepam-pretreated gerbils and controls. Since the dose of GR73632 (3 pmol/5 μl) was selected to produce a near maximal foot tapping response, it may be less amenable to attenuation by non-tachykinin NK_1 receptor antagonists. We are presently looking at lower doses of GR73632 (0.5–1 pmol/5 μl) to see if various anxiolytic/antidepressant drugs will affect this response. Interestingly, GR73632-induced vocalisations in guinea-pigs are attenuated by some (but not all) drugs belonging to this class, e.g. imipramine, fluoxetine, but not diazepam. In contrast, vocalisations in guinea-pig pups induced non-pharmacologically by maternal separation seem to be reliably blocked by a wide range of anxiolytics and antidepressant drugs, including diazepam (Kramer et al., 1998; Rupniak et al., 2000).

There was some preliminary evidence for dissociations between the effects of diazepam and the tachykinin NK_1 receptor antagonists on shock-induced defaecation. Diazepam robustly inhibited both shock-induced foot tapping and defaecation, whereas the tachykinin NK_1 receptor antagonists only blocked the foot tapping response. This might suggest that tachykinin NK_1 receptor antagonists only block certain fear-related behaviours, and perhaps not somatic signs, however, a tachykinin NK_1 receptor antagonist has been shown to decrease restraint stress-induced defaecation in the rat (Ikeda et al., 1995) and so further work is necessary to establish the generality of this result. The finding that diazepam increased immobility time was a surprise observation and seems contrary to the known anxiolytic effects of benzodiazepines. However, we feel that this likely reflects a limitation to the present technique for scoring this behaviour and does not reflect diazepam-induced increased freezing scores. Rather, it may reflect that diazepam pretreatment reduced some spontaneous behaviours in this test. Since we attempted no discrimination between measuring immobility and freezing, as discussed earlier, a more detailed ethological approach to scoring gerbil behaviour in this test may be necessary.

NK_1 receptors have been proposed to play a role in the modulation of nociception, and indeed CP-99,994 has been shown to produce an analgesic effect in mice, particularly

against the late phase of formalin-induced licking (Seguin et al., 1995). Therefore, it is possible that the tachykinin NK_1 receptor antagonists produced an analgesic effect such that the gerbils had a reduced perception of the shock. However, in all experiments, tachykinin NK_1 receptor antagonist-pretreated gerbils displayed responses to footshock that were identical to controls. Moreover, CP-99,994 has been shown to be more effective against prolonged noxious chemical stimuli and only at ataxia-producing doses does CP-99,994 block the reflexive response to mechanical and thermal noxious stimuli (Seguin et al., 1995). In addition, NK_1 receptor knockout mice do not differ in their acute nociceptive thresholds when compared to wild-type mice, which suggests that substance P does not mediate acute pain sensation (De Felipe et al., 1998).

A further potential confound to the interpretation that tachykinin NK_1 receptor antagonists reduce shock-induced foot tapping by a reduction of fear/anxiety, is that these drugs may impair the learning processes essential to form associations between the shock unconditioned stimulus and the conditioned stimulus. However, to the best of our knowledge there is no evidence to suggest that tachykinin NK_1 receptor antagonists impair learning. For instance, NK_1 knockout mice can form conditioned associations between food or an acute cocaine injection and a novel environment (Murtra et al., 2000). However, one way to empirically test this is to study the effect of tachykinin NK_1 receptor antagonists on foot tapping induced by cue reexposure (retest) after conditioning in a drug-free state. In the present study, MK869-pretreated gerbils were retested 24 h after conditioning and a similar blockade of the foot tapping response was recorded. However, this drug does have a long duration of action (Hale et al., 1998), so it is possible that the gerbils were not completely drug-free at retest.

Fluoxetine was found in these studies to actually increase foot tapping induced by footshock. This may be related to the anxiogenesis frequently reported following acute pretreatment with selective serotonin reuptake inhibitors (Van Praag, 1988; Westenberg and Den Boer, 1988; Bodnoff et al., 1989; Griebel et al., 1995). It would be interesting to establish whether this apparent potentiation remains following chronic fluoxetine treatment, for tolerance tends to develop to the acute anxiogenic effects of selective serotonin reuptake inhibitors (Bodnoff et al., 1989; Griebel et al., 1994, 1995). It would also be of value to study other anxiogenic compounds on gerbil foot tapping behaviour.

In conclusion, the present series of experiments suggest a novel approach for looking at NK_1 receptor antagonists on gerbil behaviour. An advantage of this test is that it utilises a species whose NK_1 receptor pharmacology resembles human. As yet we have only studied the effect of drugs on behaviour during the conditioning session—we have not systematically studied the effects of tachykinin NK_1 receptor antagonists on the foot tapping produced by cue reexposure in the absence of footshock, i.e. the retest session. Nonetheless, this preclinical work lends support to the proposed anxiolytic/antidepressant potential of this drug class. Future clinical studies with the various tachykinin NK_1 receptor antagonists currently in development will determine whether this potential is to be realised.

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