ELSEVIER

European Journal of Pharmacology 369 (1999) 1–9

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The thyrotrophin-releasing hormone analogue MK771 induces tic-like behaviours: the effects of dopamine D_1 and D_2 receptor antagonists

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Received 12 January 1999; accepted 19 January 1999

Abstract

Thyrotrophin-releasing hormone (TRH) and its analogues induce tic-like behaviours in rodents such as blinking and forepaw licking. Changes in spontaneous blinking frequency are observed in several disease states with dopamine abnormalities and dopaminergic agents modulate blinking. We have therefore investigated the effects of dopamine D_1 and D_2 receptor antagonists on TRH analogue (1-pyro-2-aminoadipyl-L-histidyl-L-thiazolidine-4-carboxamide; MK771)-induced blinking and bouts of forepaw licking. MK771 (2.5 mg/kg)-induced blinking was not attenuated by the dopamine D_1 receptor antagonists (+)-7-chloro-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro(1*H*)-3-benzazepine maleate (SCH23390) (0.01, 1.0 and 5.0 mg/kg) and ((−)-*trans*-6,7,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-*N*-methyl-5-*H*-benz[2,1b]azepine (SCH39166; 1.0 and 5.0 mg/kg) or the dopamine D_2 receptor antagonists raclopride (3.0 and 5.0 mg/kg) and sulpiride (5.0 and 10.0 mg/kg). D_1 but not D_2 receptor antagonists attenuated MK771-induced forepaw licking. MK771-induced blinking, therefore, appears not to involve dopamine D_1 or D_2 receptors and contrary to previously held belief dopamine does not appear to be pivotal in the control of blinking, while MK771-induced forepaw licking is modulated by dopamine D_1 but not D_2 receptors.

Keywords: Blinking; Dopamine; Thyrotrophin-releasing hormone (TRH); Forepaw licking

1. Introduction

Perturbations of spontaneous blink rates have been demonstrated in a number of neurological and psychiatric conditions. Parkinsonian patients display a characteristic reduction in spontaneous blink rates (Hall, 1945), which is correlated with the reduction in dopamine seen in the substantia nigra (Adams and Victor, 1981). Elevated blink rates have been observed as one of the first signs of Gilles de la Tourette's Syndrome (Bonnet, 1982). Neuroleptics can reduce the increased blink rates seen in schizophrenia (Karson et al., 1981 Karson et al., 1982, 1983; Kleinman et al., 1984; Muesser et al., 1984; Helms and Goodwin, 1985), an increase which is thought to be a consequence of the disease rather than of the treatment (Mackert et al., 1991). Animal studies suggest that dopamine D_2 receptor agonists, such as bromocriptine, 4-propyl-9-hydroxy naphazine and apomorphine increase blinking, and this is attenuated by dopamine D_2 receptor antagonists (Casey et al., 1980; Karson et al., 1981, 1983; Elsworth et al., 1991; Lawrence and Redmond, 1991). Dopamine D_2 receptor antagonists also reduce spontaneous blink rates and this effect can be reversed with dopamine D_2 receptor agonists (e.g., Lawrence et al., 1991). The dopamine D_1 receptor agonist dihydrexidine also induced blinking which was attenuated by the dopamine D_1 receptor antagonist SCH23390 (Elsworth et al., 1991; Taylor et al., 1991).

Thyrotrophin-releasing hormone (TRH), administered intra-cerebro-ventricularly, induced blinking which was not antagonised by the dopamine antagonist haloperidol (Dursun and Handley, 1991). Blinking has also been noted in rats after administration of TRH or TRH analogues (personal communication to A.C. McCreary from Dr. K.C.F. Fone, July 22nd, 1996).

The actions of TRH do not appear to be restricted to actions on the hypothalamo–pituitary axis. Indeed, the peptide has been detected in a number of extra-hypothalamic areas (Gilbert et al., 1982; Lighton et al., 1984;

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00039-4

Przegalinski and Jaworska, 1990; Przegalinski et al., 1990; Sasek et al., 1990) and TRH receptors have a heterogeneous distribution throughout the brain (Sharif, 1989).

Behavioural interactions of TRH and its analogues with central dopaminergic systems have been demonstrated. When TRH or the TRH analogues CG3703 or CG3705 were injected into the nucleus accumbens, locomotor activity was augmented (Miyamoto and Nagawa, 1977; Heal et al., 1981), and this was attenuated by neuroleptic administration (Miyamoto et al., 1979; Heal et al., 1981). It is thought that these locomotor effects were due to the dopamine releasing actions of TRH (Kerwin and Pycock, 1979; Sharp et al., 1982).

TRH and its analogues induce a variety of tic-like behaviours in rodents, such as head-shakes and wet-dog shakes (Wei, 1975; Heal et al., 1981; Yamada et al., 1984; Simasko and Horita, 1985; Fone et al., 1987; Johnson et al., 1989; Fone et al., 1989a,b; Dursun and Handley, 1991). The 5-HT_{2A} receptor mediated head-shake (Kennett and Curzon, 1991; Kennett et al., 1994) is thought to be a constituent of the grooming repertoire (Wei, 1981) and can be modulated by dopamine D_1 receptors (Schreiber et al., 1995; Dursun and Handley, 1996). D_1 receptor agonists induce grooming (Molloy and Waddington, 1984, 1987a; Arnt et al., 1987; Starr and Starr, 1986a,b; Eilam et al., 1992) which can be antagonised by dopamine D_1 receptor antagonists (Starr and Starr, 1986b). TRH and its analogues also induce grooming of the forepaws (forepaw licking) (Fone et al., 1987, 1989a,b; Johnson et al., 1989; McCreary and Handley, 1993, 1994, 1995a) and forepaw tremor is also observed after TRH or TRH analogue administration (Wei, 1975; Yarbrough, 1978; Costall et al., 1979; Simasko and Horita, 1985; Fone et al., 1987; McCreary and Handley, 1994, 1995a) and a combined measure of forepaw licking and forepaw tremor was not antagonised by haloperidol (Dursun and Handley, 1991).

The aims of the current study were therefore to assess the actions of selective dopamine D_1 and D_2 receptor antagonists (+)-7-chloro-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro(1*H*)-3-benzazepine maleate (SCH23390), ((−)-*trans*-6,7,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-*N*-methyl-5-*H*-benz[2,1b]azepine (SCH39166), raclopride and sulpiride (Iorio et al., 1983; Ögren et al., 1986; Chipkin et al., 1988; for review see Dursun and Handley, 1996) upon blinking and forepaw licking induced by the TRH analogue 1-pyro-2-aminoadipyl-L-histidyl-L-thiazolidine-4-carboxamide (MK771; Veber et al., 1976), that is centrally active after peripheral administration.

2. Methods

2.1. Subjects

Experimentally naive Aston bred male MF1 mice, 20–30 g in weight, housed in groups of 10 adjacent to the experimental room for at least 2 days prior to experimentation and allowed tap water and food (41B Cube Diet, Pilsbury's, Birmingham, UK) ad libitum, served as subjects in this study. The experimental and housing rooms were maintained at 21 ± 2°C, relative humidity 50–60% on a 12-h light–dark cycle (lights on 08:00–20:00 h).

2.2. Experimental sessions

All experimental sessions were performed between 08:30 and 15:00 h in glass aquaria ($25 \times 20 \times 20$ cm) with black fabric floors partitioned with black card to form small observation chambers ($20 \times 9 \times 20$ cm). Behaviour was recorded on video tape (Panasonic WV-KT115E video camera linked to a Panasonic NV-FS90B S-VHS recorder) and analysed off-line with the aid of a BBC computer (program written by Dr. T.C. Kirkham and supplied by Prof. S.J. Cooper). Each depression of a computer key led to a cumulative count of the behaviour assigned to that key. It was not always possible to observe each mouse for the full duration of the recording session, if the mouse turned away from the camera or, occasionally, was obscured by the other mouse in the chamber. Correction for this time that the mouse was not observed ('time-out') was achieved by multiplying the count for each behaviour by a correction term: [Bin Duration (5 min)] over [Bin Duration − 'time-out']. All experimental work was performed in conformity with the Animals (Scientific Procedures) Act 1986.

2.2.1. Experiment 1

Since L-5-hydroxytryptophan-induced head-shake frequency was affected by acute isolation (Boulton and Handley, 1973), both acutely isolated and pairs of mice were tested in preliminary studies.

Saline (10.0 ml/kg, i.p.) or MK771 (5.0 mg/kg, i.p.) was administered to either acutely isolated or paired mice (6 mice/treatment group) and their blink rates and bouts of forepaw licking behaviour counted in 5 min bins for 30 min immediately following injection. This dose was chosen in preliminary studies since a 5 mg/kg dose induced another tic-like behaviour in rats (Simasko and Horita, 1985). Using the data recorded from these experiments (cumulative counts over 30 min), the time course and differences between paired and isolated mice could be analysed. Subsequent experiments were performed in paired mice, since forepaw licking was more prominent in paired mice (see Section 3). The 5 min duration of observation following a 7.5 min pretreatment time was selected, since the peak of behavioural effects in paired mice occurred in this time period.

The frequency distribution of MK771-induced blinking and forepaw licking was assessed in mice from control (paired) groups (blinking, $n = 152$; forepaw licking, $n = 139$).

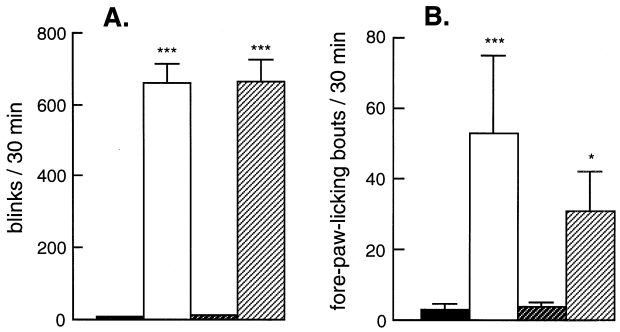


Fig. 1. (A) Data representing the total number of blinks recorded over a 30 min period following administration of MK771 (5.0 mg/kg, i.p.) to paired and acutely isolated mice and (B) the total number of forepaw licking bouts recorded over a 30 min period following administration of MK771 (5.0 mg/kg, i.p.) to paired and acutely isolated mice. ***, $P < 0.001$ and *, $P < 0.05$ cf. saline control. Closed bars, saline (acutely isolated mice); open bars, MK771 (acutely isolated mice); vertically hatched bars, saline (paired mice) and hatched bars (MK771 paired mice).

2.2.2. Experiment 2

The dose–response relationships of blinking and forepaw licking were ascertained in paired animals for a 5 min time period for 7.5 min after treatment with MK771 (0.25–60.0 mg/kg i.p.).

2.2.3. Experiment 3

Saline (10.0 ml/kg, i.p.), the dopamine D_1 receptor antagonists SCH23390 (0.01, 1.0 and 5.0 mg/kg, i.p.) and SCH39166 (1.0 and 5.0 mg/kg, i.p.) or the dopamine D_2 receptor antagonists raclopride (3.0 and 5.0 mg/kg, i.p.) or sulpiride (5.0 and 10.0 mg/kg, i.p.) were administered 22.5 min before MK771 (2.5 mg/kg, i.p.) and blinking and forepaw licking bouts were recorded 7.5 min later for a 5 min period. Doses and route of administration were based on previous studies conducted in our laboratory (Dursun and Handley, 1996).

2.3. Drugs

All drugs were dissolved in 0.9% saline vehicle and all were injected in a volume of 10.0 ml/kg. MK771 (1-pyro-2-aminoadipyl-L-histidyl-L-thiazolidine-4-carboxamide) was a gift from Merck, Sharp and Dohme (Harlow, UK). SCH23390 ((+)-7-chloro-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro(1*H*)-3-benzazepine maleate) and (±)-sulpiride were obtained from Research Biochemicals, SCH39166 ((−)-*trans*-6,7,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-*N*-methyl-5-*H*-benz[2,1b]azepine) from Schering-Plough, NJ, USA and raclopride from Astra (London, UK).

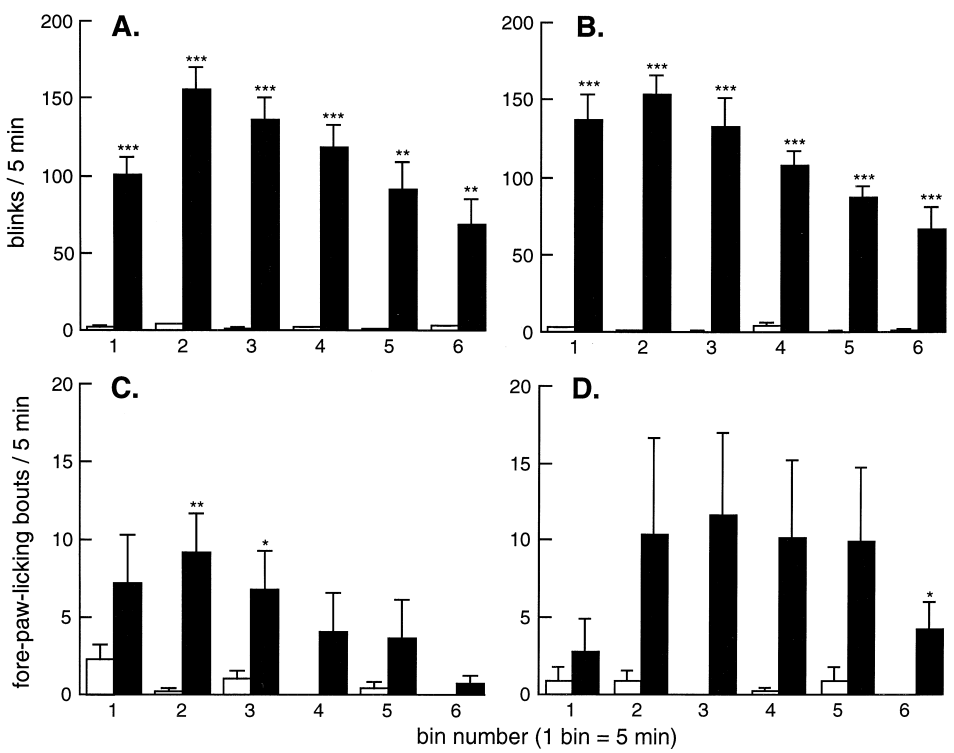


Fig. 2. (A) Data representing the total number of blinks recorded in successive 5 min bins following administration of MK771 (5.0 mg/kg, i.p.) to paired mice. (B) Data representing the total number of blinks recorded in successive 5 min bins following administration of MK771 (5.0 mg/kg, i.p.) to acutely isolated mice. (C) Data representing the total number of forepaw licking bouts recorded in successive 5 min bins following administration of MK771 (5.0 mg/kg, i.p.) to paired mice. (D) Data representing the total number of forepaw licking bouts recorded in successive 5 min bins following administration of MK771 (5.0 mg/kg, i.p.) to acutely isolated mice. Closed bars represent MK771 and open bars saline treatment. Closed bars represent MK771 and open bars saline treatment. ***, $P < 0.001$, **, $P < 0.01$ and *, $P < 0.05$ *cf.* saline control.

2.4. Statistical analyses

Experiment 1: the *cumulative counts* for blinking and forepaw licking bouts were obtained by summing each of the six 5 min time recording bins. Main treatment effects were analysed using a two-way analysis of variance (ANOVA) with post-hoc Fisher's least significant difference test. *Time course:* each of the separate treatments (saline or MK771) were compared within each 5 min time course bins using an unpaired student's *t*-test. Frequency distributions were tested for normality using a correlation test for normality (Schaeffer and Farber, 1992), whereby normal score values were correlated with behavioural counts for blinking and forepaw licking and significance of the correlation was calculated with reference to statistical tables. *Experiment 2*: the ED_{50} of the ascending portion of the MK771-induced blinking dose response function was assessed using linear regression analysis. An ED_{50} for the induction of forepaw licking was not established due to the nature of the dose response. *Experiment 3, antagonism studies* (*blinking*): main effects of pretreatment (i.e., saline vehicle or antagonist), treatment (i.e., saline vehicle or MK771) and pretreatment by treatment interactions were analysed with a two-way analysis of variance and following significant ANOVA tests, interactions between independent groups were assessed with post-hoc *t*-tests. Non-normally distributed *forepaw licking* data were analysed with a non-parametric Kruskall–Wallis test followed by post-hoc Mann–Whitney *U*-tests and Bonferonni correction for multiple comparisons as appropriate. Statistical difference from control was pre-defined at the $P < 0.05$ level.

3. Results

MK771 induced a behavioural syndrome consisting of blinking, bouts of forepaw licking and of forepaw tremor (unilateral and bilateral expressions were not differentiated on counting); tail elevation and tremor along with rapid vertical movements of the tail which presented with contractions of abdominal musculature, ear scratch, hyperlocomotion and infrequent back-muscle contractions. Headshakes were not observed after MK771 administration.

3.1. Experiment 1

Significant main effects of treatment were observed on blinking ($F(1,20) = 273.1$, $P < 0.0001$; Fig. 1, panel A), whereas there were no significant main effects of condition (i.e., acutely isolated or paired mice) ($F(1,20) = 0.003$, $P < 0.9544$) or treatment × condition interaction ($F(1,20) = 0.007$, $P < 0.9362$). Significant main effects of treatment ($F(1,20) = 9.39$, $P < 0.0061$; Fig. 1, panel B), but not condition ($F(1,20) = 0.003$, $P < 0.9544$) or treatment × condition interaction ($F(1,20) = 0.007$, $P < 0.9362$) were observed on forepaw licking. Post-hoc analyses revealed that MK771 induced blinking and forepaw licking in both acutely isolated and paired mice (see Fig. 1 for significance levels).

MK771 induced significant increases in blinking in paired mice in each of the six 5 min bins recorded which peaked in the 2nd and 3rd bins (5–10 min and 10–15 min following injection) (Fig. 2, panel A). In acutely isolated mice, significance from vehicle controls was also seen at all time points, but the blink rate peaked in the 1st and 2nd bins (Fig. 2, panel B).

Forepaw licking was significantly increased in paired animals by prior treatment with MK771 during bins 2 and 3 (5–10 min and 10–15 min after injection) (Fig. 2, panel C), but significance was only observed during bin 6 (25–30 min after administration of MK771) in acutely isolated mice (Fig. 2, panel D).

Frequency distribution analyses of MK771-induced blinking revealed a significant normal distribution ($P < 0.01$; correlation coefficient = 0.995). However, MK771-induced forepaw licking behaviour was not normally distributed (not significant; correlation coefficient = 0.934).

3.2. Experiment 2

The dose–response relationship for MK771-induced blinking was bell-shaped, $ED_{50} = 1.72$ [1.38–3.63] mg/kg, i.p.) for the ascending linear portion of the curve (Fig. 3, panel A). The number of MK771-induced forepaw licking bouts appeared not to be related to the dose administered over the dose range tested (Fig. 3, panel B).

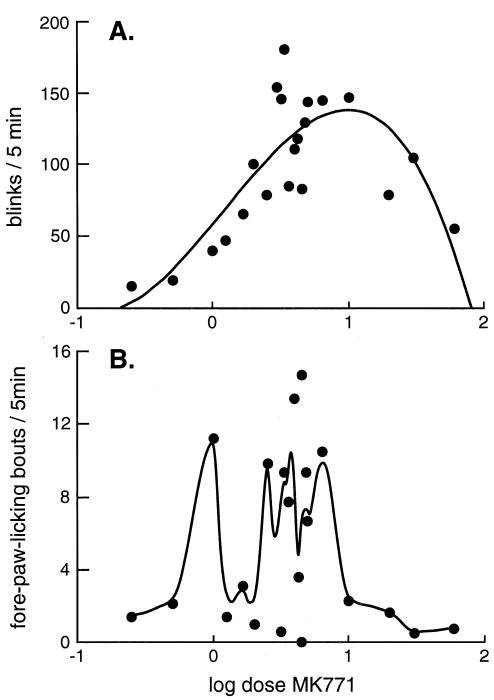
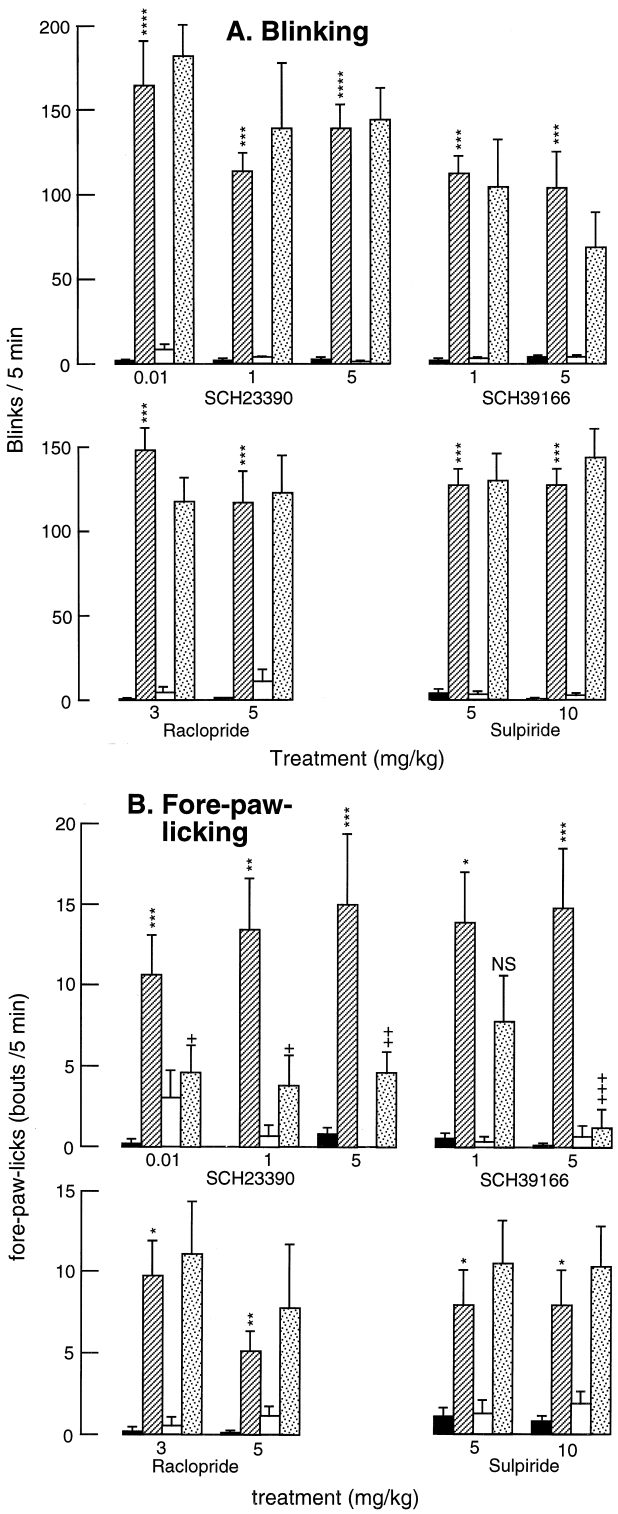


Fig. 3. (A) Data representing the bell-shaped dose–response curve for MK771-induced blinking and (B) the lack of a dose–response relationship for MK771-induced forepaw licking.

3.3. Experiment 3

No significant alteration in basal activity blinking or forepaw licking was observed after pretreatment of any of the antagonists tested (Fig. 4, panels A and B).



3.3.1. SCH23390

SCH23390 (0.01 mk/kg) failed to modify MK771-induced blinking. Two-way ANOVA revealed a significant main effect of MK771 (2.5 mg/kg) treatment ($F(1,83) = 155$, $P < 0.0001$), but no significant main effect of pretreatment or pretreatment vs. treatment interaction (Fig. 4, panel A). Non-parametric Kruskall–Wallis analyses of forepaw licking revealed a significant effect of the groups tested ($P < 0.0001$), post-hoc analyses (Mann–Whitney *U*-test with Bonferonni correction) demonstrated that MK771 induced forepaw licking and these effects were attenuated by SCH23390 (0.01 mg/kg, $P < 0.01$; 1.0 mg/kg, $P < 0.01$ and 5.0 mg/kg, $P < 0.01$).

3.3.2. SCH39166

SCH39166 (1.0 and 5.0 mg/kg) failed to modify MK771-induced blinking. Two-way ANOVA revealed a significant main effect of MK771 (2.5 mg/kg) treatment ($F(1,68) = 64.39$, $P < 0.001$), but no significant main effect of pretreatment or pretreatment vs. treatment interaction (Fig. 4, panel A). Kruskall–Wallis analyses of forepaw licking revealed a significant effect of the groups tested ($P < 0.0001$), post-hoc analyses (Mann–Whitney *U*-test with Bonferonni correction) demonstrated that MK771 induced forepaw licking ($P < 0.0001$) and these effects were attenuated by SCH39166 (5.0 mg/kg, $P < 0.001$), the lower dose (1.0 mg/kg, $P < 0.11$) failed to reach significance.

3.3.3. Raclopride

Raclopride (3.0 and 5.0 mg/kg) was without effect on MK771-induced blinking. Two-way ANOVA revealed a significant main effect of MK771 (2.5 mg/kg) treatment ($F(1,44) = 137$, $P < 0.0001$), but no significant main effects of pretreatment or pretreatment vs. treatment interaction were observed (Fig. 4, panel A). Analyses of forepaw licking revealed a significant effect of treatment ($P < 0.025$) and post-hoc tests confirmed that MK771 induced forepaw licking.

3.3.4. Sulpiride

Sulpiride (10.0 and 15.0 mg/kg) was without effect on MK771-induced blinking. Two-way ANOVA revealed a

Fig. 4. Data showing the effects of the dopamine D_1 and D_2 antagonists SCH23390, SCH39166, raclopride and sulpiride on MK771-induced blinking (A) and forepaw licking (bouts) (B). None of the antagonists tested modulated blinking. SCH23390 (0.01, 1.0 and 5.0, mg/kg) and SCH39166 (5.0 mg/kg i.p.) significantly attenuated MK771-induced forepaw licking. Sulpiride and raclopride were without effect on MK771-induced blinking and forepaw licking. MK771-induced behaviours significantly different from saline + saline controls are signified by **** $P < 0.0001$, *** $P < 0.001$ and ** $P < 0.01$ and significance from saline + MK771 groups by $^{+++}$ $P < 0.001$, $^{++}$ $P < 0.01$ and NS, not significant. Filled bars represent saline + saline controls; hatched bars, saline + MK771; open bars, antagonist + saline and stippled bars antagonist + MK771 treated mice.

significant main effect of MK771 (2.5 mg/kg) treatment ($F(1,38) = 94.71$, $P < 0.001$), but no significant main effects of pretreatment or pretreatment vs. treatment interaction were observed, Fig. 4, panel A. Analyses of forepaw licking revealed a significant effect of treatment ($P < 0.001$) and post-hoc tests confirmed that MK771 induced forepaw licking ($P < 0.01$).

4. Discussion

The TRH analogue MK771 induced a behavioural syndrome in mice similar to that observed in previous studies (Fone et al., 1987, 1989a,b; Johnson et al., 1989; Dursun and Handley, 1991), except that head-shaking was not seen. It is unclear why this was the case, since head-shakes have been observed in the same strain of mice after intra-cerebral ventricular administration of TRH-amide (Dursun and Handley, 1991) and wet-dog-shakes have been noted in the rat after treatment with MK771 (Simasko and Horita, 1985). It is possible that the number of mice present in each observation chamber may have contributed to this, since when the number of mice per observation chamber is reduced there is a reduction in the frequency of the L-5-hydroxytryptophan-induced head-shake (Boulton and Handley, 1973). However, the differences reported when mice were reduced from two to one per observation chamber in the head-shake response (Boulton and Handley, 1973) were not mirrored with MK771-induced blinking and forepaw licking. An increase in the variability for forepaw licking was seen in acutely isolated mice. Thus, subsequent studies were performed in paired mice and the 7.5–12.5 min time window after MK771-administration was used.

Dose response analyses revealed a bell-shaped dose response curve for MK771 induced blinking, but forepaw licking was not dose-related. No previous dose–response studies have been performed upon TRH or TRH analogue-induced blinking but the forepaw licking results are consistent with the lack of a dose response relationship to the TRH analogue CG3509 (Johnson et al., 1989). However, when induced by the TRH analogues CG3703 and RX77368 forepaw licking tended to increase with dose (Johnson et al., 1989). It was surprising that sulpiride, raclopride, SCH23390 and SCH39166 failed to suppress MK771-induced blinking, given the known involvement of dopamine in spontaneous blinking (Casey et al., 1980; Karson et al., 1981, 1983; Karson, 1983; Lawrence and Redmond, 1991; Elsworth et al., 1991; Taylor et al., 1991). However, haloperidol was without action upon TRH-induced blinking (Dursun and Handley, 1991). Thus, it is apparent from the latter study and results presented here that presumed TRH receptor mediated blinking is not related to dopamine receptor mediated blinking, since pretreatment with the dopamine D_1 receptor antagonists SCH23390 and SCH39166 and dopamine D_2 receptor antagonists raclopride and sulpiride were without effect on MK771-induced blinking. It is therefore suggested that the population(s) of receptors involved in TRH receptor stimulated blinking may not be under the control of central dopamine D_1 or dopamine D_2 receptors. It is expected, by reference to the literature, that the doses of antagonists used here were sufficiently high (Karson, 1983; Starr and Starr, 1986a,b; Dursun and Handley, 1996).

In contrast to the lack of effect on blinking, pretreatment with SCH39166 and SCH23390 significantly attenuated forepaw licking bouts. This suggests that dopamine released as a consequence of TRH receptor stimulation might then act on dopamine D_1 receptor population(s) mediating these effects. The locus of the action is unclear since modulation of dopamine release following TRH or TRH analogue administration has been demonstrated in a number of different nuclei (Plotnikoff et al., 1972; Plotnikoff et al., 1975; Agarwal et al., 1977; Rastogi et al., 1981; Sharp et al., 1982; Annunziato et al., 1984; Crespi et al., 1986; Xu et al., 1990; Nishikawa et al., 1993).

Prior studies have reported that systemic and intrathecal administration of TRH analogues induces forepaw licking (Fone et al., 1987, 1989a,b; Johnson et al., 1989) which is attenuated by α-adrenoceptor and 5-HT receptor (Fone et al., 1987, 1989a; Johnson et al., 1989; McCreary and Handley, 1993, McCreary and Handley, 1994), see later. Forepaw licking might be considered as part of the grooming repertoire and dopamine D_1 receptors are involved in grooming (Molloy and Waddington, 1984; Starr and Starr, 1986a,b; Arnt et al., 1987; Molloy and Waddington, 1987a; Eilam et al., 1992), however the role of dopamine D_2 receptors cannot be excluded (Navarro et al., 1997). The low dose of SCH23390 (0.01 mg/kg) failed to increase forepaw licking compared with vehicle treated controls, as previously reported for grooming behaviour (Starr and Starr, 1986a,b). The D_2 receptor antagonists raclopride and sulpiride were without effect on forepaw licking. The lack of inhibitory effect is consistent with the failure of haloperidol to attenuate TRH induced forepaw licking/tremor and blinking in mice (Dursun and Handley, 1991). However, haloperidol and chlorpromazine attenuated TRH and TRH analogue-induced locomotor activity (Heal et al., 1981). Sulpiride has been reported to enhance intense grooming (Waddington et al., 1986; Molloy and Waddington, 1987b), whereas dopamine D_2 receptor agonists inhibit grooming (Starr and Starr, 1986a,b; Linthorst et al., 1992; Eilam et al., 1992). However, no significant enhancement of MK771-induced forepaw licking was observed with the dopamine D_2 receptor antagonists used.

It is unclear which receptor is involved in MK 771-induced blinking. TRH-induced blinking was attenuated by the 5-HT_2 receptor antagonist ritanserin (1 mg/kg; but not higher doses) and by ketanserin (Dursun and Handley, 1991; McCreary and Handley, 1995b) and is antagonised by low, but not high doses of the 5-HT_{1A} receptor agonists 8-hydroxy-2-(di-*n*-aminopropyl)tetralin (8-OH DPAT) and

buspirone (McCreary and Handley, 1992). It has been suggested that the α_1-adrenoceptors may mediate TRH or analogue-induced forepaw licking (e.g., Fone et al., 1987; McCreary and Handley, 1994), although the same is not true for blinking (McCreary and Handley, 1994). Moreover, 5-HT_{2A} and 5-HT_{1A} receptors may be involved in MK 771-induced forepaw licking, since ritanserin, ketanserin, 8-OH DPAT and buspirone but not the putative 5-$HT_{2B/2C}$ receptor antagonist SB 200646A attenuated MK 771-induced forepaw licking (McCreary and Handley, 1993, 1994, 1995a).

In summary, administration of the TRH analogue MK771 resulted in the induction of blinking and forepaw licking. Blinking was not modulated by either dopamine D_1 or D_2 receptor antagonists and therefore appears to be pharmacologically distinct from dopamine mediated blinking. In contrast, the involvement of dopamine D_1 receptors in MK771-induced forepaw licking is suggested. Dopamine D_2 receptor antagonism failed to modulate MK771-induced forepaw licking, suggesting that this receptor subtype does not modulate MK771-induced forepaw licking.

Acknowledgements

We would like to thank Merck, Sharp and Dohme, Astra Pharmaceuticals and Schering-Plough for the kind gift of drugs used in this study, Prof. S.J. Cooper for the kind gift of the behavioural analysis program and Mr. Graham Smith for his assistance with the preparation of the figures.

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