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Involvement of 5-HT_{2A} receptors in the elevation of rat serum corticosterone concentrations by quipazine and MK-212

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Abstract

The possible involvement of 5-HT_{2A} or 5-HT_{2C} receptors in the elevation of serum corticosterone in rats by quipazine (2-(1-piperazinyl)quinoline maleate) and MK-212 (6-chloro-(1-piperazinyl)pyrazine), direct-acting 5-HT receptor agonists, was investigated by the use of two newly available receptor antagonists, SB 200646A (*N*-(1-methyl-5-indolyl)-*N'*-(3-pyridyl)urea) and MDL 100,907 (*R*-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidinemethanol). MDL 100,907 blocked the increase in serum corticosterone elicited by quipazine and MK-212 with ED_{50} values of 0.0028 and 0.0027 mg/kg, s.c., respectively. In contrast, SB 200646A only partially antagonized the serum corticosterone concentration increases by quipazine and MK-212 even at the highest dose tested, 40 mg/kg, i.p. Because published data show the affinities of MDL 100,907 and SB 200646A for 5-HT_{2C} receptors to be nearly identical, whereas the affinity of MDL 100,907 for 5-HT_{2A} receptors is 17 500-fold higher than that of SB 200646A, our findings suggest that 5-HT_{2A} receptors rather than 5-HT_{2C} receptors mediate the serum corticosterone increases by both quipazine and MK-212.

Keywords: 5-HT_{2A} receptor; 5-HT_{2C} receptor; Quipazine; MK-212; MDL 100,907; SB 200646A; Corticosterone, serum; (Rat)

1. Introduction

Serotonin (5-hydroxytryptamine; 5-HT) is one of many neurotransmitters that influence hypothalamic control of pituitary function. 5-HT-containing nerve terminals make synaptic connections with corticotropin-releasing factor-containing neurons in the paraventricular nucleus of the rat hypothalamus (Liposits et al., 1987). 5-HT stimulates the release of corticotropin-releasing factor from the hypothalamus, corticotropin-releasing factor stimulates the release of adrenocorticotropin from the anterior pituitary gland, and adrenocorticotropin stimulates release of corticosterone from the adrenal cortex (Tuomisto and Mannisto, 1985; Fuller, 1992). The hypothalamic-pituitary-adrenocortical axis in rats is activated by various serotoninergic drugs, including direct- and indirect-acting 5-HT receptor agonists, 5-HT uptake inhibitors, 5-HT releasers and the 5-HT precursor, L-5-hydroxytryptophan (Fuller, 1990; Fuller and Snoddy, 1990).

Direct-acting 5-HT receptor agonists may increase serum corticosterone concentration in rats by activating receptors of either the 5-HT_{1A} or 5-$HT_{2A/2C}$ subtypes (Koenig et al., 1987; Lorens and Van de Kar, 1987; Przegalinski et al., 1989; Fuller et al., 1978; Fuller and Snoddy, 1979; Fuller, 1980). While it seems clear that activation of the 5-HT_{1A} receptor subtype can increase pituitary-adrenocortical secretion in rats, the elucidation of which 5-HT_2 receptor subtype – 5-HT_{2A} or 5-HT_{2C} – activates the hypothalamic-pituitary-adrenocortical axis in rats has been difficult due to the lack of drugs able to distinguish between these receptors. Many 5-HT receptor agonists, including quipazine (2-(1-piperazinyl)quinoline maleate) and MK-212 (6-chloro-(1-piperazinyl)pyrazine), have affinity for both 5-HT_{2A} and 5-HT_{2C} receptor subtypes, as do most antagonists which block their effects (Conn and Sanders-Bush, 1987; for review, see Fuller, 1992).

SB 200646A (*N*-(1-methyl-5-indolyl)-*N'*-(3-pyridyl)urea) is the first selective 5-HT_{2C} receptor antagonist reported to have a 50-fold higher affinity for 5-HT_{2C} receptors than for 5-HT_{2A} receptors (Forbes et al., 1993; Kennett et al., 1994). Another serotonergic agent, MDL 100,907 (*R*-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidinemethanol), is a selective 5-HT_{2A} receptor antagonist reported to have a 300-fold greater affinity for 5-HT_{2A} receptors than for 5-HT_{2C} receptors

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(Dudley et al., 1990; Palfreyman et al., 1993). While SB 200646A and MDL 100,907 have similar affinities for the 5-HT_{2C} receptor, their affinities for the 5-HT_{2A} receptor differ approximately 17 500-fold. Using these selective 5-HT receptor antagonists, we investigated the receptor subtype mediating the increase in rat serum corticosterone concentration elicited by quipazine and MK-212.

2. Methods

Male Sprague-Dawley rats (190–220 g) were purchased from Charles River Breeding Laboratory (Portage, MI). Rats were housed in groups of 5 in a 22°C room with lights on from 07:00 to 19:00 h for 1 week prior to experimentation. Food and water were freely available. SB 200646A (*N*-(1-methyl-5-indolyl)-*N'*-(3-pyridyl)urea) was a gift from SmithKline Beecham Pharmaceuticals (West Sussex, UK) and was dissolved in 25% 2-hydroxypropyl-β-cyclodextrin (Research Biochemicals, Natick, MA) for injection at 2 ml/kg (i.p.). MDL 100,907 (*R*-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol) was a gift from Marion Merrell Dow Research Institute (Cincinnati, OH) and was dissolved in 0.01 N hydrochloric acid for injection at 1 ml/kg (s.c.). Quipazine maleate (2-(1-piperazinyl)quinoline maleate) was purchased from Miles Laboratories (Elkhart, IN) and was dissolved in distilled water for injection at 1 ml/kg (s.c.). MK-212 (6-chloro-(1-piperazinyl)pyrazine) was a gift from Merck Sharp and Dohme Research Laboratories (Rahway, NJ) and was dissolved in 0.01 N HCl for injection at 1 ml/kg (s.c.). All control rats received vehicle injections. After treatment rats were decapitated between 09:00 and 10:00 h, trunk blood was collected and allowed to clot. Serum was obtained by centrifugation and stored frozen prior to assay. Serum corticosterone concentrations were measured by radioimmunoassay (Corticosterone ^{3}H-Kit, ICN Biomedicals, Inc. (Costa Mesa, CA)). Samples were diluted according to kit instructions and analyzed in duplicate. Statistical analyses were done by analysis of variance using Tukey's Honestly Significant Difference method ($P \leq 0.05$) based on the mean square error.

3. Results

Fig. 1 shows the effect of pretreatment with MDL 100,907 (15 min) on the quipazine-induced increase in rat serum corticosterone concentration at 1 h. MDL 100,907 by itself, at doses as high as 3 mg/kg, s.c., had no effect on rat serum corticosterone (data not shown). Quipazine alone significantly increased corticosterone concentrations. MDL 100,907 pretreatment antagonized the quipazine-induced increase in rat serum corticosterone concentrations in a dose-dependent manner. The dose of MDL 100,907

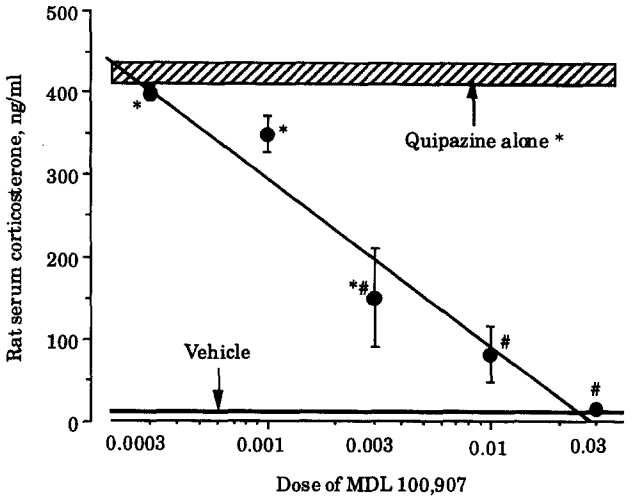

Fig. 1. MDL 100,907 antagonism of the quipazine-induced increase in rat serum corticosterone. MDL 100,907 was injected s.c. at the doses shown 15 min prior to vehicle (bottom bar) or 2.5 mg/kg, s.c. quipazine maleate (top bar). Rats were killed 1 h after quipazine injection. Means and standard error for 5 rats per group are shown (* $P \leq 0.05$ vs. vehicle control; # $P \leq 0.05$ vs. quipazine alone).

required to antagonize the increase in corticosterone by quipazine 50% (ED_{50}) was 0.0028 mg/kg, s.c.

Fig. 2 shows the effect of pretreatment with MDL 100,907 (15 min) on the increase in rat serum corticosterone concentration 1 h after MK-212. MDL 100,907 antagonized the MK-212-induced increase in rat serum corticosterone concentrations in a dose-related manner with an ED_{50} of 0.0027 mg/kg, s.c.

Fig. 3 shows the effect of SB 200646A pretreatment (15 min) on the quipazine-induced increase in rat serum cor-

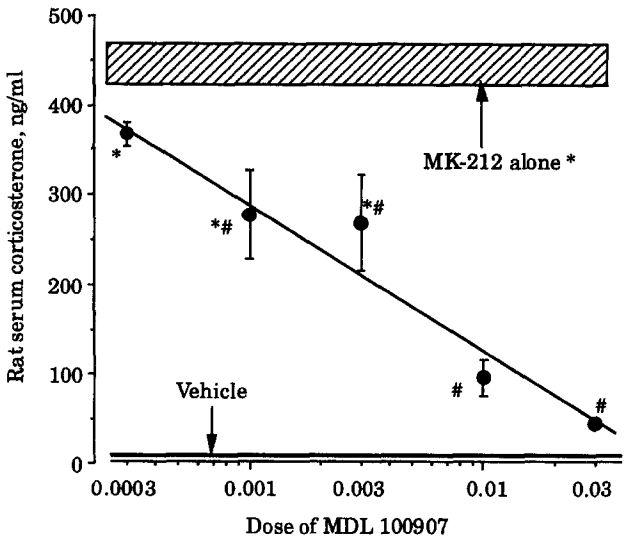

Fig. 2. MDL 100,907 antagonism of the MK-212-induced increase in rat serum corticosterone. MDL 100,907 was injected s.c. at the doses shown 15 min prior to vehicle (bottom bar) or 3 mg/kg, s.c. MK-212 (top bar). Rats were killed 1 h after MK-212 injection. Means and standard error for 5 rats per group are shown (* $P \leq 0.05$ vs. vehicle control; # $P \leq 0.05$ vs. MK-212 alone).

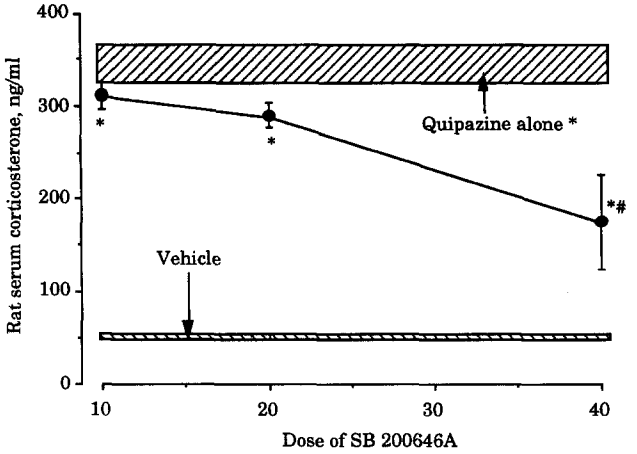

Fig. 3. SB 200646A antagonism of the quipazine-induced increase in rat serum corticosterone. SB 200646A was injected i.p. at the doses shown 15 min prior to vehicle (bottom bar) or 2.5 mg/kg, s.c. quipazine maleate (top bar). Rats were killed 1 h after quipazine. Means and standard errors for 5 rats per group are shown (* $P \leq 0.05$ vs. vehicle control; # $P \leq 0.05$ vs. quipazine alone).

ticosterone concentrations at 1 h. In this experiment, SB 200646A was injected alone at doses of 10, 20 and 40 mg/kg and failed to alter serum corticosterone concentrations (data not shown). Quipazine administration significantly increased rat serum corticosterone concentrations over control values. Pretreatment with SB 200646A at 10 and 20 mg/kg, i.p., had no effect on the quipazine-induced increase of corticosterone concentrations. At 40 mg/kg, i.p., SB 200646A significantly antagonized the increase in corticosterone elicited by quipazine, but only partial antagonism occurred (58%).

Fig. 4 shows the effect of SB 200646A pretreatment (15 min) on the MK-212-induced increase in rat serum corticosterone concentrations at 1 h. MK-212 alone significantly increased corticosterone concentrations. Pretreatment with 10 and 20 mg/kg, i.p. SB 200646A had no effect on the increase in corticosterone concentrations by MK-212. At 40 mg/kg, i.p., SB 200646A significantly antagonized the increase in rat serum corticosterone concentration elicited by MK-212, but only partially (34%).

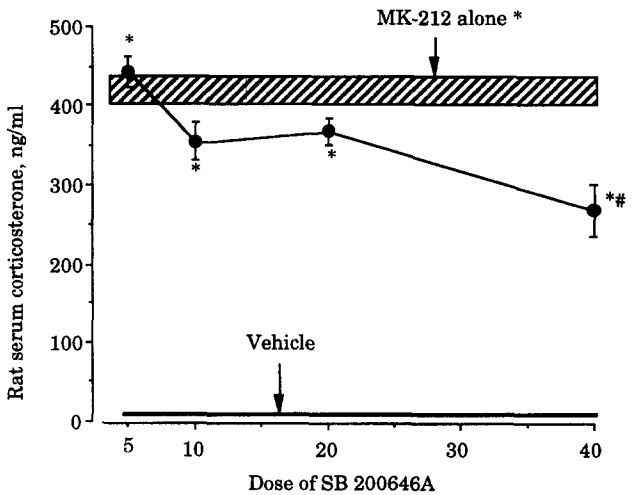

Fig. 4. SB 200646A antagonism of the MK-212-induced increase in rat serum corticosterone. SB 200646A was injected i.p. at the doses shown 15 min prior to vehicle (bottom bar) or 2.5 mg/kg, s.c. MK-212 (top bar). Rats were killed 1 h after MK-212. Means and standard errors for 5 rats per group are shown (* $P \leq 0.05$ vs. vehicle control; # $P \leq 0.05$ vs. MK-212 alone).

4. Discussion

The increases in rat serum corticosterone concentrations elicited by quipazine and MK-212 are thought to be mediated by direct activation of 5-HT_{2A} or 5-HT_{2C} receptors (Fuller et al., 1978; Fuller and Snoddy, 1979, 1984; Fuller, 1980; Fuller and Mason, 1986; Conn and Sanders-Bush, 1987; Koenig et al., 1987; Nash et al., 1988). This conclusion is not based on the selectivity of quipazine or MK-212 for these receptor subtypes, but rather on the receptor selectivity of various antagonists. Many of these antagonists fail to discriminate between 5-HT_{2A} and 5-HT_{2C} receptors (Leysen et al., 1982; Hoyer et al., 1986).

There has been previous disagreement about which 5-HT_2 receptor subtype mediates activation of the pituitary-adrenocortical axis by MK-212. Koenig et al. (1987) suggested that 5-HT_2 (now called 5-HT_{2A}) receptors mediated corticosterone increases by MK-212 in rats based on antagonism by ketanserin, ritanserin, altanserin and metergoline. That same year, Lorens and Van de Kar (1987) suggested that 5-HT_{2A} receptors did not mediate corticosterone increases by MK-212 because LY53857 did not block those increases. Later, King et al. (1989) postulated that 5-HT_{1C} (now called 5-HT_{2C}) receptors mediated adrenocorticotropin increases by MK-212 in rats, based on antagonism by mesulergine and metergoline, but not by spiperone, ketanserin or pindolol.

The 5-HT_{2A} receptor has generally been implicated in activation of the pituitary-adrenocortical axis by quipazine, as the corticosterone elevation by quipazine was blocked by many 5-HT_{2A} receptor antagonists, including some having relatively less affinity for 5-HT_{2C} receptors, e.g., spiperone, ketanserin and MDL 11,939 (Fuller and Snoddy, 1979, 1984, 1990; Koenig et al., 1987; Fuller, 1990, 1991, 1992).

The previous literature then leaves open the possibility that different receptor subtypes mediate pituitary-adrenocortical activation by quipazine and MK-212. If separate receptors can be proven to mediate these effects of quipazine and MK-212, then antagonism of their actions could be a useful measure of in vivo efficacy and selectivity of antagonists. The present data using two newly developed receptor antagonists suggest that corticosterone elevation by quipazine and MK-212 is mediated by the same receptor and that it is the 5-HT_{2A} receptor.

MDL 100,907 is a selective antagonist of 5-HT_{2A} recep-

tors (K_i = 0.36 nM) vs. 5-HT_{2C} receptors (K_i = 105 nM) in radioligand binding studies in vitro (Dudley et al., 1990; Palfreyman et al., 1993). Local perfusion of MDL 100,907 (1–10 nM) has been shown electrophysiologically to reverse 5-HT receptor activation of interneurons in rat piriform cortex by at least 50% (Marek and Aghajanian, 1994). MDL 100,907 has also been reported to antagonize 1-(2,5-demethoxy-4-iodophenyl)-2-aminopropane (DOI)-induced head twiches in mice with an ED_{50} = 0.03 mg/kg, i.p. (Dudley et al., 1990). These latter effects of MDL 100,907 are thought to be mediated by blockade 5-HT_{2A} receptors and occur in the same dose range. The potency of MDL 100,907 can only be compared in a limited way, however, since different species and routes of administration were used.

SB 200646A is the first antagonist reported to show marked selectivity for the 5-HT_{2C} receptor subtype (K_i = 126 nM) versus the 5-HT_{2A} receptor subtype (K_i = 6319 nM) in radioligand binding studies in vitro (Forbes et al., 1993; Kennett et al., 1994). SB 200646A at doses from 10 to 40 mg/kg, p.o. has been shown to antagonize 1-(3-chlorophenyl)piperazine (mCPP)-induced hypolocomotion, hypophagia in food-deprived rats and anxiety in the social interaction test, consistent with 5-HT_{2C} receptor blockade. SB 200646A had no effect on 1-(2,5-demethoxy-4-iodophenyl)-2-aminopropane (DOI)-induced head shakes at doses as high as 200 mg/kg, p.o., suggesting a lack of 5-HT_{2A} receptor antagonism (Kennett et al., 1994).

MDL 100,907, along with SB 200646A, are thus useful tools that were not available at the time King et al. (1989) did their studies to identify the 5-HT_2 receptor subtype responsible for the increase in rat serum corticosterone concentrations by quipazine and MK-212. The affinity of SB 200646A for the 5-HT_{2C} receptor subtype (K_i = 126 nM) is nearly identical to the affinity of MDL 100,907 for the 5-HT_{2C} receptor subtype (K_i = 105 nM). In contrast, the two antagonists have very different affinities for the 5-HT_{2A} receptor subtype. MDL 100,907 (K_i = 0.36 nM) has 17 500 times higher affinity than SB 200646A (K_i = 6319 nM) for the 5-HT_{2A} receptor (Forbes et al., 1993; Dudley et al., 1990; Palfreyman et al., 1993). Our data show that MDL 100,907 is more than 10 000 times more potent than SB 200646A in antagonizing the increase in rat serum corticosterone after quipazine or MK-212 administration. These findings overall suggest that both quipazine and MK-212 increase rat serum corticosterone concentrations by activating 5-HT_{2A} receptors.

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