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Pharmacological Evaluation of the Histamine H_1 and 5-HT Blocking Properties of 2-*N*-(Carboxamidinonormianserin) (FCC5): in-vitro studies

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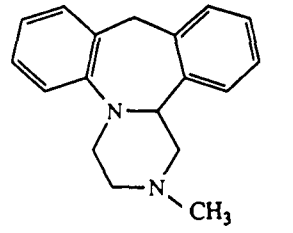
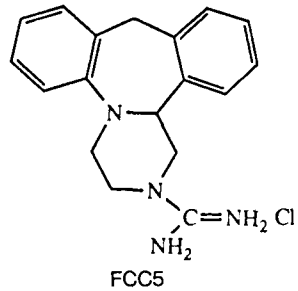
Abstract—Some in-vitro pharmacological effects of a novel analogue of mianserin, 2-carboxamidino-1,2,3,4,10,14b-hexahydrodibenzo (c,f) pyrazino (1,2,-α) azepine hydrochloride (FCC5) have been studied. FCC5 was a non-competitive antagonist of both histamine-induced contractions of the guinea-pig ileum and 5-HT-induced contractions of rat fundal strips with pD'_2 values of 6·13 and 5·57, respectively. The insurmountable antihistaminic effect of FCC5, 100 nM, in the guinea-pig isolated ileum was not removed by washing. FCC5, 10–100 nM, had no effect on responses to acetylcholine or barium chloride of the guinea-pig isolated ileum. In guinea-pig isolated right atria, FCC5, 1–30 μM, had no effect on H_2-receptor-mediated chronotropic responses to histamine. FCC5, 10–1000 nM, had no α_2-adrenoceptor antagonist activity, as assessed by lack of effect on the inhibitory responses to B-HT 920 in the electrically stimulated rat isolated vas deferens. FCC5 resembles mianserin by being a potent, non-competitive antagonist at histamine H_1 and 5-HT receptors, but differs from mianserin in a number of respects including having much less effect at α_2-adrenoceptors.

2-Carboxamidino-1,2,3,4,10,14b-hexahydrodibenzo (c,f) pyrazino (1,2,-α) azepine hydrochloride (FCC5) (Fig. 1) is a novel histamine and 5-HT receptor antagonist with no detectable central nervous system (CNS) effects in laboratory animals (Leitch et al 1990). The pharmacological profile of FCC5 has been characterized in-vivo in a number of test preparations (Rechtman et al 1989). FCC5 was found to (i) have sympathomimetic properties in anaesthetized cats, rats and guinea-pigs, possibly due to blockade of neuronal uptake of noradrenaline, (ii) lack antagonist activity at α_1- and α_2-adrenoceptors in the anaesthetized and pithed rat preparations, and (iii) inhibit histamine and leukotriene release from human- and guinea-pig-sensitized lung fragments (Temple et al 1992).

In the present study the pharmacological properties of FCC5 were examined using a number of in-vitro preparations. Comparisons were made with its parent analogue, mianserin (Fig. 1) and with certain more specific and potent antagonists where appropriate.



Mianserin



FCC5

FIG. 1. The structure of FCC5 and mianserin.

Materials and Methods

Effects of FCC5, mianserin and mepyramine on responses of guinea-pig ileum to histamine

Male guinea-pigs, 500–600 g, were killed instantly by cervical dislocation and exsanguination. An approximate 2 cm length of ileum was removed and mounted in a 20 mL organ bath containing Tyrode solution (bubbled with 95% O_2–5% CO_2) of the following composition (mM): NaCl 136·89, KCl 2·68, $CaCl_2$ 1·80, $MgCl_2$ 1·05, NaH_2PO_4 0·42, $NaHCO_3$ 11·90 and dextrose 5·55. The tissue was equilibrated for 45–60 min under 0·5 g resting tension before drugs were administered.

Initial experiments were performed to examine the time course of the onset of action of drugs and persistence of their effects after removal by washing. After equilibration in Tyrode solution at 32°C, five contractile responses of the ilea to histamine (3 μM, producing responses 60–90% of maximum) were obtained every 3 min leaving the histamine in contact with the tissue for 30–45 s. The bathing solution was then changed to Tyrode solution containing either FCC5, mianserin, mepyramine (all at 0·1 μM) or an equivalent volume of vehicle. A response to the same concentration of histamine was obtained 15 min later. The bathing solution was changed back to Tyrode solution free from the antago-

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nist and responses to histamine again determined every 15 min for the following 2 h (with further washing). Responses to histamine in the absence or presence of FCC5, mianserin or mepyramine were expressed as a percentage of the mean of the five initial responses.

In subsequent experiments (32°C), concentration response curves were generated by adding histamine cumulatively (Van Rossum 1963) at 30–45 s intervals to the bath. An initial concentration response curve was followed by a second, defined as the control. After the ileum had been incubated with the antagonist for 15 min, a third concentration response curve was obtained. Using mepyramine, subsequent concentration response curves in the presence of higher concentrations were performed after washing the ileum and restoration of the baseline tension. However, because of the persistent actions of FCC5 and mianserin (see below), fresh pieces of ileum were used to examine effects of higher concentrations. The mean contractile response to each concentration of histamine, in the control and in the presence of the various concentrations of the antagonist, was expressed as a percentage of the control maximum response. The pD'_2, the negative logarithm of the concentration of the antagonist which produced a 50% inhibition of the maximum contractile response, was calculated for FCC5 and mianserin according to the procedure of Van Rossum (1963). The pA_2 for mepyramine was determined from a Schild plot (Arunlakshana & Schild 1959). Similar experiments were performed using acetylcholine and barium chloride as agonists.

Effects of FCC5, mianserin and ranitidine on chronotropic responses of guinea-pig right atria to histamine

Guinea-pig right atria were removed after killing (as described above) and mounted in a 20 mL organ bath containing Krebs-Henseleit solution (32°C, bubbled with 95% O_2–5% CO_2) of the following composition (mM): NaCl 118, KCl 4·70, $CaCl_2$ 2·54, KH_2PO_4 1·18, $MgSO_4$ 1·17, $NaHCO_3$ 24·88, glucose 5·55. Atria were equilibrated under 0·5 g resting tension for 45–60 min before drugs were administered.

Increases in the rate of atrial contractions were measured (beats min^{-1}) in response to cumulatively increasing concentrations of histamine. The initial concentration-response curve to histamine was defined as the control. Each atrium was then incubated for 15 min with the vehicle or a concentration of the antagonist being studied, and responses to cumulative concentrations of histamine were again measured. Subsequent concentration-response curves in the presence of higher concentrations of the antagonist were obtained only after washing to restore the basal atrial rate. Positive chronotropic responses to histamine were expressed as an increase in the rate of atrial contractions (beats min^{-1}).

Effects of FCC5 and mianserin on responses to 5-HT of rat isolated stomach fundus strips

Male Wistar rats, 150–300 g, were killed by cervical dislocation and the pyloric antrum of the stomach removed. Strips 4–5 cm long were obtained by opening each fundus along its lesser curvature, preserving the longitudinal musculature (Vane 1957). A strip 2 cm in length and approximately 2 mm wide, was mounted in a 20 mL organ bath containing Krebs-Henseleit solution (as above, 37°C, bubbled with 95% O_2–5% CO_2). Each strip was under 1·0 g resting tension and equilibrated for 45–60 min before starting experiments.

Concentration response curves were obtained by adding 5-hydroxytryptamine (5-HT) cumulatively (Van Rossum 1963) to the organ bath. An initial control concentration-response curve was obtained. The strip was then incubated for 20 min with the antagonist being studied, and a second concentration-response curve to 5-HT obtained. Subsequently, using separate stomach strips, concentration-response curves to 5-HT in the presence or absence of higher concentrations of the antagonists were obtained similarly. Mean contractile responses were expressed as a percentage of the mean control maximum response to 5-HT. The pD'_2 value was calculated for FCC5 or mianserin.

Effects of FCC5 and mianserin on the inhibitory effects of B-HT 920 on rat isolated transmurally stimulated vas deferens

Male Wistar rats, 150–300 g, were killed by cervical dislocation. Both vasa deferentia were isolated and mounted individually in 20 mL organ baths containing Krebs-Henseleit solution (composition as above, 36°C, bubbled with 95% O_2–5% CO_2). Each vas deferens was equilibrated at a resting tension of 0·5 g for 30–45 min before commencing electrical field stimulation, by means of two platinum electrodes, one placed parallel alongside and the other inserted from the bottom vertically inside the lumen. Stimulus parameters were 0·2 Hz with square pulses of 1 ms duration at a supramaximal voltage (70 V).

Concentration response curves were generated by adding B-HT 920 cumulatively at 1 min intervals. The initial concentration-response curve to B-HT 920 was defined as the control. After the vas deferens had been incubated with the antagonist for 15 min, responses to cumulative concentrations of B-HT 920 were again determined. Higher concentrations of antagonist were studied on the same tissue after washing and restoration of the basal twitch response. Responses were expressed as a percentage of the maximum inhibitory response, obtained in the control. The data were used to generate a plot according to Arunlakshana & Schild (1959).

Time control studies

Parallel experiments in which the four types of tissues (as described above) did not receive any antagonist were performed in order to monitor any time-dependent changes in agonist sensitivity.

Grass force-displacement transducers (FT03) and polygraph (79E) were used to record contractions of all isolated tissues.

Drugs

FCC5 (Fig. 1) was prepared in the Department of Organic Chemistry at Monash University (Jackson et al 1992). Other compounds used were: acetylcholine chloride, histamine diphosphate, 5-hydroxytryptamine creatinine sulphate (Sigma); B-HT 920 dihydrochloride (Boehringer Ingleheim); mepyramine maleate (May and Baker); methysergide hydrogen maleate (Sandoz); mianserin hydrochloride (Research Biochemicals Inc). Saline (NaCl, 0·9% w/v) was used as the vehicle for all drugs.

Statistical analysis

Data are expressed as mean ± s.e. m; where appropriate Student's paired or unpaired *t*-tests were used for comparison of two means. A probability value of less than 0·05 was considered as significant. Log concentration-response curves were analysed by regression analysis over the linear portion of the curves (Diem & Leutner 1970).

Results

Effect of FCC5, mianserin and mepyramine on responses of guinea-pig ileum to histamine

Fig. 2 shows the persistence during washing of the antagonism of submaximal histamine responses by FCC5, mianserin and mepyramine following a 15 min contact time. The inhibitory effects of equiconcentrations of all antagonists were substantial after 15 min contact with the tissue. On removal of the antagonist by washing, the effects of mepyramine were reversible during the following 60–90 min whereas those of FCC5 were not, and those of mianserin were only partially so.

FCC5 (10–100 nM) had no effect on the concentration-response curves to acetylcholine or barium chloride in the guinea-pig isolated ileum (n = 4, data not shown).

As shown in Fig. 3A and C, in the presence of FCC5 (1–1000 nM) or mianserin (1–100 nM) the log concentration response curves to histamine were shifted to the right, and with high concentrations, maximum responses were depressed. In contrast, in the presence of mepyramine (1–100 nM, Fig. 3B) a parallel rightward shift occurred of the log concentration response curve with no effect on the maximum responses.

pD'_2 values for FCC5 and mianserin were calculated to be 6·13 ± 0·12 (n = 8) and 6·94 ± 0·14 (n = 8), respectively. The pA_2 for mepyramine was calculated to be 8·9 (8·42–10·00, 95% confidence limits; slope of Schild plot 0·93 (0·45–1·4); n = 4).

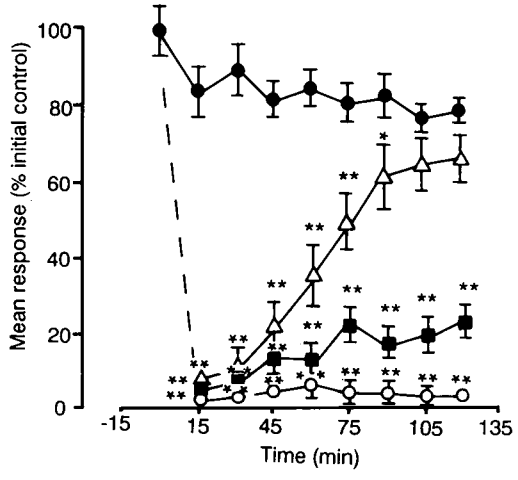

FIG. 2. Time course for the onset and recovery during washing of the antihistaminic effect of 100 nM mepyramine, FCC5 or mianserin (15 min contact) in the guinea-pig ileum. ●, Histamine (3 μM) time controls; ○, after FCC5; ■, after mianserin; △, after mepyramine. Points are mean and vertical lines s.e., n = 8. $*P < 0.05$, $**P < 0.01$, compared with corresponding controls.

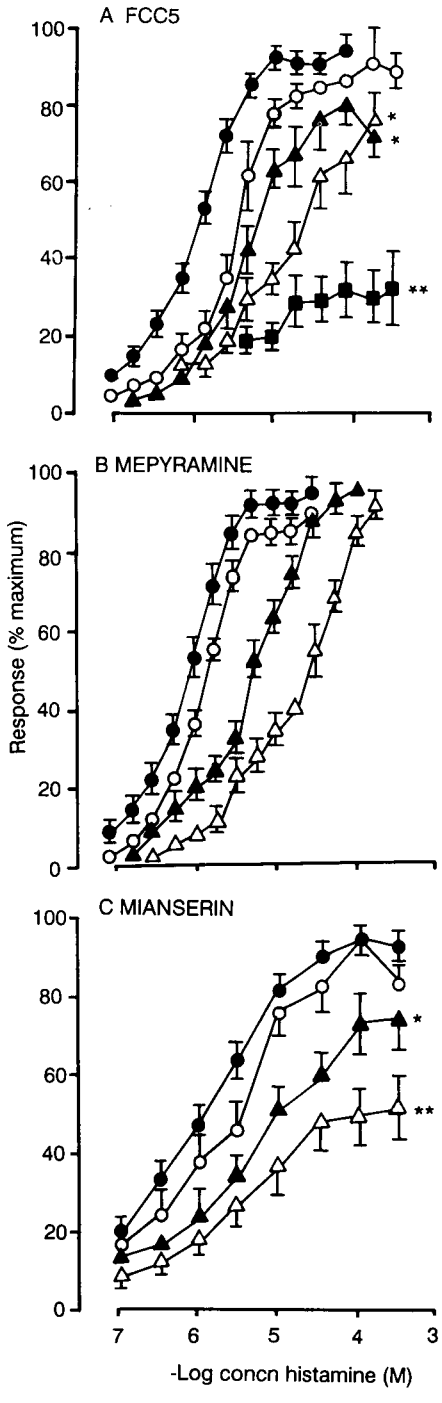

FIG. 3. Effects of FCC5, mepyramine and mianserin on responses to histamine of guinea-pig ileum. Control histamine curve ●, responses following (A) FCC5, (B) mepyramine or (C) mianserin; 1 nM ○; 10 nM ▲; 100 nM △ and 1000 nM ■, are shown. Points are mean and vertical lines s.e., n = 4–8. $*P < 0.05$, $**P < 0.01$ compared with corresponding control.

Effects of FCC5, mianserin and ranitidine on chronotropic responses of guinea-pig right atria to histamine

As shown in Fig. 4C, increasing concentrations of ranitidine caused a concentration-related displacement to the right of the histamine concentration-response curve without significantly depressing the maximum response, indicative of

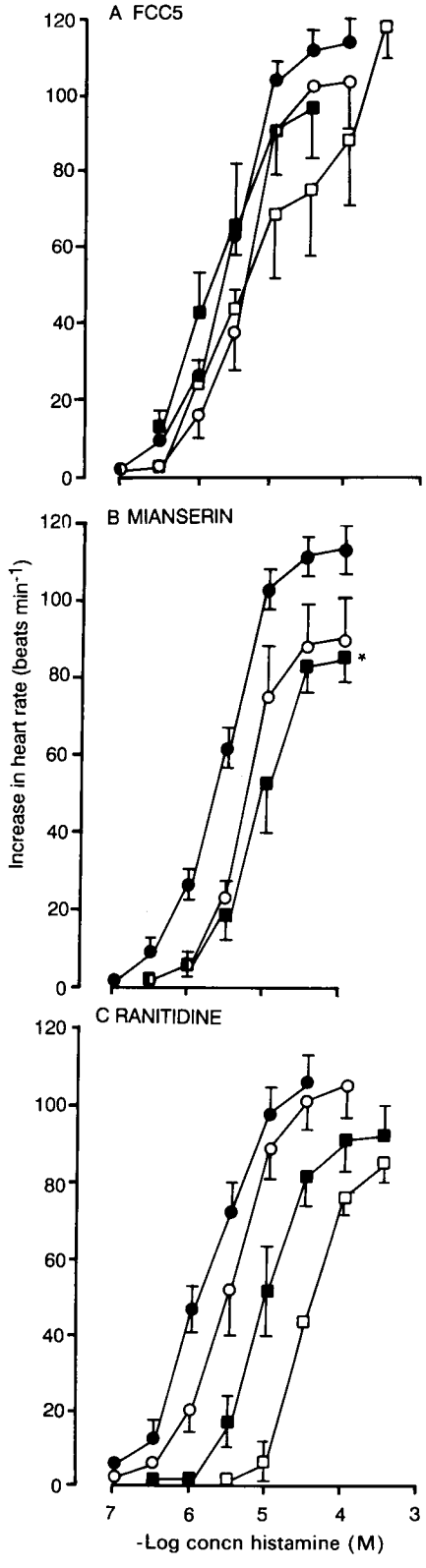

FIG. 4. Effects of FCC5, mianserin or ranitidine on chronotropic responses to histamine of guinea-pig right atria. Control histamine curve ●, responses following (A) FCC5: 1 μM ○, 10 μM ■, 30 μM □; (B) mianserin: 10 μM ○, 30 μM ■; or (C) ranitidine: 0·1 μM ○, 1 μM ■ and 3 μM □ are shown. Points are mean and vertical lines s.e., n = 4–8. *$P < 0.05$, compared with corresponding control.

competitive antagonism. High concentrations of mianserin (10–30 μM) also shifted the concentration-response curve to the right (degree of shift at 30 μM was calculated to be 3·0 ± 0·56-fold; n = 5), but with depression of the maximum (Fig. 4B). In contrast, FCC5 (1–30 μM) had no significant effect on the histamine concentration-response curve. The pA_2 value for ranitidine was calculated to be 6·93 (6·65–7·96, 95% confidence limits; slope of Schild plot 1·14 (0·46–1·81); n = 6).

Effects of FCC5 and mianserin on responses to 5-HT of rat isolated stomach fundus strips

The presence of either FCC5 (1–1000 nM) or mianserin (1–1000 nM) caused rightward shifts of the 5-HT log concentration-response curves with the higher concentrations causing reduced maximum responses (Fig. 5).

pD'_2 values of 5·57 ± 0·10 (n = 8) and 5·39 ± 0·15 (n = 8) were found for FCC5 and mianserin, respectively.

Effects of FCC5 and mianserin on the inhibitory effects of B-HT 920 on rat isolated transmurally stimulated vas deferens

As shown in Fig. 6A, FCC5 (10–1000 nM) had no effect on the ability of B-HT 920 to inhibit electrically-induced

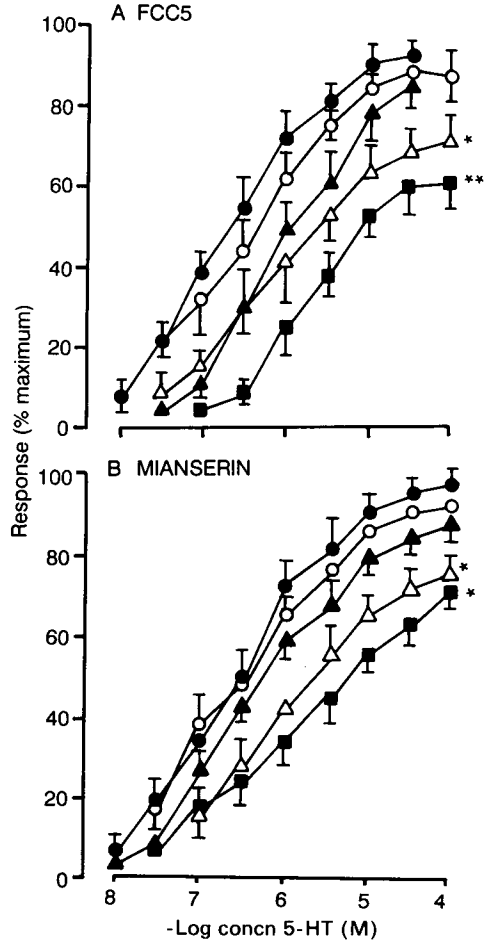

FIG. 5. Effects of FCC5 or mianserin on responses to 5-HT of rat stomach strip. Control 5-HT curve ●, responses after (A) FCC5 or (B) mianserin; 1 nM ○; 10 nM ▲; 100 nM △ and 1000 nM ■ are shown. Points are mean and vertical lines s.e., n = 4–10. *$P < 0.05$, **$P < 0.01$, compared with corresponding control.

twitches. In contrast, mianserin (10–1000 nM) shifted the log concentration-response curve to the right in a parallel manner without affecting maximum responses (Fig. 6B). The pA_2 for mianserin was 6·70 (6·36–7·34, slope of Schild plot 1·02 (0·68–1·37); n = 5).

High concentrations of FCC5 (10 μM) significantly reduced ($P < 0.05$) the height of the twitch response to electrical stimulation to 49·4 ± 7·6% of the control twitch responses (n = 5). In contrast, an identical concentration of mianserin potentiated significantly ($P < 0.01$) twitch responses to 191·2 ± 12·2% of control twitch responses (n = 5) (data not shown).

Time control studies

In all four isolated tissue preparations used, no significant time-dependent changes of the agonist concentration response curves occurred when compared with control curves (n = 4–6, data not shown).

Discussion

These results demonstrate that in-vitro FCC5 resembles its close structural analogue mianserin by being a potent and relatively specific antagonist of histamine and 5-HT with, however, some differences. FCC5 resembled mianserin by inhibiting contractile responses to histamine of the guinea-pig isolated ileum mediated by H_1-receptors (Ash & Schild 1966). Low concentrations of both drugs caused parallel shifts to the right in the histamine log concentration-response curves with higher concentrations causing reduction in their slopes and maximum responses. This suggests that both FCC5 and mianserin are non-competitive histamine H_1-receptor antagonists. Calculation of the pD'_2 values for each drug indicated that FCC5 was less potent than mianserin at depressing maximum responses to histamine. The antagonism caused by FCC5 also resembled that of mianserin and contrasted with that of mepyramine by showing persistence. Antagonism by either FCC5 or mianserin was difficult to reverse by washing. Mepyramine in contrast acted as a competitive antagonist of histamine and its effect was readily reversible.

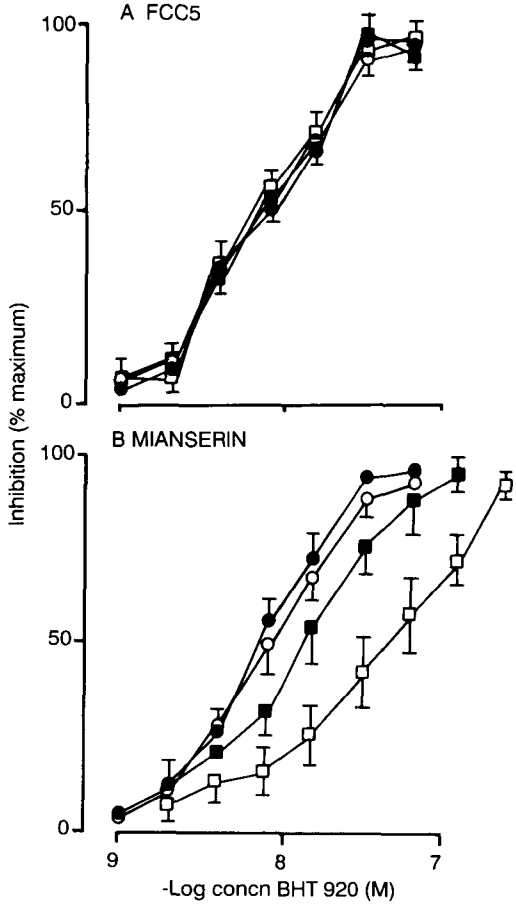

FIG. 6. Effects of FCC5 or mianserin on inhibition caused by B-HT 920 of the electrically-stimulated rat vas deferens. Control B-HT 920 inhibitory curve ●, responses after (A) FCC5 or (B) mianserin; 10 nM ○; 100 nM ■ or 1000 nM □ are shown. Points are mean and vertical lines s.e., n = 3–5.

Results obtained using the rat stomach strip indicated that FCC5 additionally resembled mianserin by being a non-competitive antagonist of 5-HT. Both drugs caused rightward shifts of the log concentration-response curves to 5-HT with significantly reduced maxima. Comparison of their pD'_2 values indicated that FCC5 and mianserin were not significantly different in their effectiveness at depressing maximum responses to 5-HT. The contractile effect of 5-HT on the rat stomach strip is thought to be mediated mainly by 5-HT_{1C}-receptors (Buchheit et al 1986), although more recent evidence may suggest otherwise (Baez et al 1990).

Evidence for the relative specificity of the antihistamine and 5-HT effects of FCC5 was provided by the finding that FCC5 (10–100 nM) had no effect on responses of the guinea-pig ileum to acetylcholine or barium chloride. Mianserin is known to be devoid of anticholinergic activity both in-vitro and in-vivo (Van Riezen et al 1981). FCC5 also had no effect at histamine H_2-receptors indicated by its lack of ability to antagonize the positive chronotropic effects of histamine in the spontaneously beating right atrium of the guinea-pig. This was in contrast to mianserin which displayed weak histamine H_2-receptor blocking properties. The potent H_2-receptor antagonist, ranitidine, demonstrated the properties of competitive antagonism in this preparation confirming the results of Black et al (1972).

FCC5 did not block α_2-adrenoceptors. This is in contrast to mianserin which is known to act as an antagonist at these receptors (Brown et al 1980). High concentrations of FCC5 did not affect inhibition caused by B-HT 920, a selective α_2-adrenoceptor agonist (Kobinger & Pichler 1981), in the rat transmurally stimulated vas deferens. However, mianserin readily antagonized the effects of B-HT 920. The dissimilarity between the two drugs was further emphasized by the finding that whereas high concentrations of mianserin potentiated responses of the rat vas deferens to electrical stimulation (α_2-adrenoceptor blockade), FCC5 caused inhibition, possibly due to its ability to block neuronal uptake of noradrenaline (Rechtman et al 1989). The neuronal uptake blocker cocaine has also been shown to inhibit responses to electrical stimulation of the rat vas deferens (Brown et al 1980).

These in-vitro data therefore demonstrate that incorporation of the guanidine moiety into the tetracyclic ring system

of mianserin retains the specific, potent and highly persistent histamine and 5-HT receptor-blocking properties whilst, at the same time, reducing its ability to block α_2-adrenoceptors. The higher basicity of FCC5 also bestows on the molecule in-vivo, in comparison with mianserin, reduced ability to penetrate the blood-brain barrier with consequently less prominent central actions (Leitch et al 1990).

In summary, FCC5, a novel tetracyclic analogue of mianserin, is a potent, non-competitive histamine H_1 and 5-HT-receptor antagonist.

Acknowledgements

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