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In-Vivo Pharmacological Studies of 2-*N*-Carboxamidinonormianserin, a Histamine and 5-Hydroxytryptamine Antagonist Lacking Central Effects

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Abstract—The in-vivo pharmacological properties have been examined of FCC5 (2-*N*-carboxamidino-1, 2, 3, 4, 10, 14b-hexahydrodibenzo (c.f.) pyrazino (1, 2-α)azepine hydrochloride), a guanidino analogue of mianserin. FCC5 (30–100 μg kg^{-1}, i.v.) caused long-lasting (>1 h) attenuation of histamine- and 5-hydroxytryptamine (5-HT)-induced bronchoconstriction in the anaesthetized guinea-pig. FCC5 (≤1 mg kg^{-1}, i.v.) had no effect on submaximal bronchoconstrictor responses caused by i.v. acetylcholine or the thromboxane A_2-mimetic U46619 ((15S)-hydroxy-11α,9α-(epoxymethano)prosta-5Z,13E-dienoic acid). The pressor effects of 5-HT in anaesthetized and pithed rats were inhibited by FCC5 (0·3–1·0 mg kg^{-1}, i.v.). Higher doses of FCC5 (3 mg kg^{-1}, i.v.) reduced bradycardia and depressor responses to 5-HT in anaesthetized rats. In anaesthetized cats and rats and also pithed rats, FCC5 (0·1–1·0 mg kg^{-1}, i.v.) caused sympathomimetic effects as demonstrated by pressor responses and tachycardia. FCC5 (0·1–0·3 mg kg^{-1}, i.v.) inhibited pressor responses to tyramine whereas those to noradrenaline and sympathetic nerve stimulation were potentiated. Oedema in the rat paw caused by intraplantar 5-HT was inhibited by FCC5 (ID50 0·76 mg kg^{-1}, i.p.; and 2·7 mg kg^{-1}, p.o.). In decerebrate rats which had been spinalized at T6-8, fenfluramine-induced facilitation of the flexor reflex of the anterior tibialis muscle was inhibited by mianserin (ID50 0·36 mg kg^{-1}, i.p.) but not by FCC5 (≤3 mg kg^{-1}, i.p.). Head twitches in carbidopa-pretreated mice induced by L-5-hydroxytryptophan were inhibited by mianserin (ID50 0·11 mg kg^{-1}, i.p.), but not by FCC5 (≤30 mg kg^{-1}, i.p.). It is concluded that FCC5 possesses antihistamine and anti-5-HT properties and is orally effective. No evidence was found for central effects.

FCC5 [Monash University: 2-*N*-carboxamidino-1, 2, 3, 4, 10, 14b-hexahydrodibenzo (c.f.) pyrazino (1,2-α) azepine hydrochloride or 2-*N*-carboxamidinonormianserin] (Fig. 1) is one of a series of recently synthesized analogues of the antidepressant, mianserin, a combined H_1, 5-HT_2 and α_2-adrenoceptor antagonist (Van der Burg et al 1970; Vargaftig et al 1971). It differs from mianserin in that the guanidine group makes it a tetracyclic compound of higher basicity. The structure of FCC5 and some of its in-vitro properties have already been described (Leitch et al 1992).

In the present investigation, the peripheral actions of FCC5 were studied in a range of in-vivo tests designed to evaluate its potency and selectivity. Experiments were performed to ascertain whether its systemic administration affected CNS-mediated responses. The pharmacological profile of FCC5 was compared with that of mianserin.

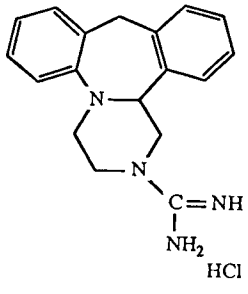

FIG. 1. The structure of FCC5.

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Materials and Methods

Chemistry

FCC5 was synthesized as part of a synthetic programme aimed at finding a potent combined antihistamine/anti-5-hydroxytryptamine (5-HT) compound with greater selectivity of action than current antihistamines (Jackson et al 1992). Mianserin, with its broad range of both central and peripheral activities was chosen for chemical modification.

FCC5 has a guanidino group substituted onto the mianserin molecule at the 2-*N* atom (Leitch et al 1992). The incorporation of this highly polar (and basic) group, which has a pK_a of ~14 and is extensively protonated at physiological pH, seemed appropriate since it would prevent penetration into the CNS and has been used in a number of successful peripherally acting drugs such as bethanidine (Boura et al 1962) and guanethidine (Maxwell et al 1960).

Bronchoconstriction in the guinea-pig

A modification of the Konzett & Rossler (1940) technique was used. Male Monash strain guinea-pigs, 0·5–1·0 kg, were anaesthetized with sodium pentobarbitone (60–120 mg kg^{-1}, i.p.). The trachea was cannulated and connected to a ventilator for artificial respiration of the animal (55 strokes min^{-1}, 10 mL kg^{-1}), after further i.p. doses of pentobarbitone (10 mg kg^{-1}) were given to inhibit spontaneous respiratory movements. Inflow pressure was measured by using a Gould-Statham P23 transducer connected to the ventilator circuit by a T-piece.

The right common carotid artery was cannulated and connected to a Gould-Statham P23 transducer so enabling blood pressure and heart rate to be recorded on a Grass

polygraph (model 79D). Rectal temperature was maintained at 37°C.

The left jugular vein was cannulated for drug administration. The bronchoconstrictor drugs used were 5-HT, acetylcholine, histamine and the thromboxane A_2-mimetic U46619 (Coleman et al 1980). Drugs were administered in volumes of 0·05–0·5 mL, and cannulae flushed with 0·2 mL of 0·9% NaCl (saline). Experiments with U46619 and acetylcholine were carried out in animals anaesthetized with urethane (25%, 10 mL kg^{-1}, i.p.)

Dose response curves were obtained to histamine (4–64 μg kg^{-1}, i.v.) and repeated after each of a series of cumulative doses of mepyramine (1–10 μg kg^{-1}, i.v.), mianserin (3–30 μg kg^{-1}, i.v.), FCC5 (3–30 μg kg^{-1}, i.v.) or equivalent volumes of saline (0·5 mL), only one antagonist or saline was given to each animal in a group.

In another group of animals, dose-response curves were obtained to 5-HT (2–64 μg kg^{-1}, i.v.) and repeated after each of a series of cumulative doses of mianserin (3–30 μg kg^{-1}, i.v.), FCC5 (10–100 μg kg^{-1}, i.v.), or equivalent volumes of saline (0·5 mL).

5-HT-induced hindpaw oedema in the rat

Male Wistar rats, 140–275 g, were fasted overnight but were allowed free access to water up to 30 min before experiments.

Each animal was lightly anaesthetized with 2% halothane in 1:1 N_2O/O_2 during injection of the plantar surface and measurement of paw volume. The latter was measured using a mercury plethysmometer (Winter et al 1962). The plethysmometer was connected to a Gould pressure transducer, Model P231D. Output from the transducer was recorded on a Grass polygraph (model 79D). Recordings were calibrated in terms of mL displacement of mercury.

Paw volumes were determined before drug administration. Hind paw oedema was induced by injection of 5-HT (10 μg) in 0·1 mL sterile saline into the subplantar tissue of the left hind paw, using a 26G needle. The same volume of sterile saline was injected into the contralateral paw of each rat. FCC5 (as a suspension in aqueous solution of 1% Tween 80) was administered 30 min (i.p.) or 60 min (p.o.) before 5-HT injection. Control groups received 1% Tween 80 (i.p. or p.o.). Any swelling of the hindpaw due to 5-HT was measured 30 min after the 5-HT injection. The ID50 for FCC5 was determined as the dose that caused a 50% reduction in paw swelling when compared with the corresponding control group.

Effects on the cardiovascular system of the rat

Chloralose-anaesthetized rats. Anaesthesia was induced in male Wistar rats, 260–360 g, with 2–4% halothane in 1:1 N_2O/O_2. The trachea was cannulated. The left jugular vein was cannulated and anaesthesia maintained with chloralose (80 mg kg^{-1}, i.v.). Rectal temperature was maintained at 37°C. Arterial blood pressure was recorded from the right carotid artery and integrated heart rate triggered from the arterial pressure wave. Both parameters were displayed on a Grass polygraph (model 79D). Drugs were injected in catecholamine diluent (0·156 g NaH_2PO_4 and 0·04 g ascorbic acid L^{-1} of saline). FCC5 was dissolved in saline, acidified with 1–2 drops of 1 M HCl and flushed into the rat with 0·25 mL saline.

Pithed rats. Male Wistar rats, 275–385 g, were anaesthetized (2–4% halothane in 1:1 N_2O/O_2). The trachea was cannulated. Each animal was pithed (Gillespie et al 1970) by passing a pithing rod through the orbit and down the spinal canal. The carotid artery was ligated and artificial ventilation carried out for the remainder of the experiment (2 mL/100 g, 55 strokes min^{-1}). Arterial blood pressure and heart rate were recorded and drugs injected through a cannulated jugular vein. Rectal temperature was maintained at 37°C. Atropine sulphate (1 mg kg^{-1}, i.v.) and (+)-tubocurarine chloride (1 mg kg^{-1}, i.v.) were administered to inhibit voluntary and parasympathetic nerve-mediated responses. Each animal was adrenalectomized before pithing.

Where indicated, the sympathetic outflow was stimulated at T6–T7 from the pithing rod which incorporated electrodes mounted 2·5 mm apart and approximately 1 cm from its distal end. Electrical stimulation was carried out for 30 s every 5–10 min at a supramaximal voltage (90 V) with pulses of 0·5 ms in duration over the frequency range of 0·3–10 Hz.

The effects of i.v. FCC5 on pressor responses to 5-HT, tyramine, noradrenaline and electrical stimulation of the sympathetic outflow were measured on a Grass polygraph (model 79D). The effects of FCC5 on the inhibitory actions of guanethidine on pressor responses to electrical stimulation of the sympathetic outflow were also studied. Dose response or frequency response curves were obtained before and after administering increasing incremental doses of FCC5. For time control studies, the vehicle was injected at similar times to FCC5.

Experiments using the anaesthetized cat

Cats of either sex, 2·5–3·9 kg, were anaesthetized with 2–4% halothane in 1:1 N_2O/O_2. The left femoral vein was cannulated and chloralose (60 mg kg^{-1}) injected i.v., after which the gaseous anaesthetic was discontinued. The left femoral artery was cannulated, and connected to a transducer for recording blood pressure with a Grass polygraph (model 79D). Heart rate was simultaneously recorded using a tachograph triggered by the arterial pressure wave.

A tracheal cannula was inserted and respiratory depth and rate monitored by a transducer connected by a T-piece to this cannula.

The right nictitating membrane was connected by a thread to an isometric transducer (Grass FTO3C) under a resting tension of 1 g. The right preganglionic cervical sympathetic and vagus nerves were separated and electrodes placed under the sympathetic nerve bathed in liquid paraffin–saline. The nerve was stimulated for 1 min periods at 0·1–10 Hz (pulse duration 0·6 ms) and at a supramaximal voltage, usually 5 V.

Loose ligatures were placed around both carotid arteries and the carotid occlusion reflex elicited by clamping the carotids bilaterally for 1 min.

Actions on the CNS

Flexor reflex in the spinal and decerebrate rat. The method used was a modification of that described by Maj et al (1976) as previously described by Rawlow & King (1991). Male Wistar rats, 250–300 g, were anaesthetized with halothane (4% halothane in 1:1 N_2O/O_2), spinalized at T6–8 by transecting the spinal cord with scissors, tracheotomized and

decerebrated with a pithing rod down to Cl-2. Each rat was ventilated at a rate of 50 strokes min^{-1}, 3 mL per stroke. Blood pressure was monitored from the right carotid artery with a pressure transducer connected to a Grass recorder (model 79B). The left carotid was ligated.

The tendon of the left anterior tibialis muscle was exteriorized and attached to an isometric transducer under a resting tension of 2 g. Reflex twitches of the anterior tibialis muscle were evoked by electrical impulses (7–20 V, 100 ms min^{-1}) delivered transcutaneously by a pair of needle electrodes to the ipsilateral hind paw. Twitches were recorded isometrically on a Grass polygraph (model 79D).

Body temperature was maintained at 36–37°C. Fenfluramine (2 mg kg^{-1}, i.v.) was administered into the tail vein 30 min after decerebration to cause facilitation of the flexor reflex for the duration of the experiment.

FCC5 or mianserin was suspended in a 1% aqueous solution of Tween 80 and administered i.p. 30 min after fenfluramine. Control animals were treated with the vehicle. The ID50 for each drug was determined as the dose that caused a 50% reduction of reflex activity facilitated by fenfluramine.

L-5-Hydroxytryptophan-induced head twitches in mice. The method used was a modification of that described by Corne et al (1963). Albino-Swiss male mice, 25–40 g, were randomly assigned to groups of five, and pretreated with FCC5 (1–30 mg kg^{-1}, i.p.), mianserin (0·1–10 mg kg^{-1}, i.p.) or vehicle (1% aqueous solution of Tween 80) together with carbidopa (25 mg kg^{-1}, i.p.). L-5-hydroxytryptophan (280 mg kg^{-1}) was administered i.p. 15 min later. Head twitches induced by L-5-hydroxytryptophan were counted during a 2 min period, 28 min after its injection. The ID50 for each drug was determined as the dose that caused a 50% reduction in the number of head twitches when compared with the control group.

Effects in conscious cats. FCC5 or bethanidine was injected s.c. into male or female cats in various doses. The animals were observed for behavioural changes for the first 7 h and then daily for the following week. Any relaxation of the nictitating membrane was noted. The pupillary reflex to light and the pinna reflex were also tested.

Drugs

FCC5 (2-*N*-carboxamidino-1, 2, 3, 4, 10, 14b-hexahydrodibenzo (c.f.) pyrazino (1, 2,-α) azepine hydrochloride) (Fig. 1), was synthesized in the Department of Organic Chemistry at Monash University. Other compounds used were: acetylcholine chloride, clonidine hydrochloride, histamine diphosphate, 5-hydroxytryptamine creatinine sulphate, noradrenaline, L-5-hydroxytryptophan (Sigma, USA), McNeil-A-343 (McNeil Laboratories, USA), methoxamine hydrochloride, mianserin hydrochloride (Research Biochemical Inc, USA), mepyramine maleate (May and Baker, UK), U46619 (15S)-hydroxy-11α, 9α-(epoxymethano)prosta-5Z,13E-dienoic acid (Upjohn, USA), tyramine monohydrochloride (Calbiochem, USA), fenfluramine (Riker laboratories, Australia), carbidopa (Merck, USA), (+)-tubocurarine chloride, atropine sulphate monohydrate (Koch Light laboratories, UK), urethane (ethyl carbamate, Ajax chemicals, Australia), pentobarbitone (David Bull, Australia), halothane (ICI Australia), chloralose (BDH, UK), guanethidine sulphate (Ciba Geigy, Australia) and bethanidine sulphate (Burroughs-Wellcome, UK). Saline was used (unless otherwise specified) as the vehicle for drugs.

Statistical analysis

Data are expressed as mean ± s.e.; where appropriate, Student's paired or unpaired *t*-tests were used for comparison of two means. $P < 0.05$ was considered significant. Log concentration-response curves were analysed by regression analysis over the linear portion of the curves (Diem & Leutner 1970).

Results

Bronchoconstriction in the guinea-pig

Bronchoconstrictor responses of the anaesthetized guinea-pig to histamine (4–64 μg kg^{-1}, i.v.) were attenuated by mepyramine, mianserin, and FCC5 (Table 1). In contrast to mepyramine, high doses of FCC5 produced non-parallel shifts of histamine log dose-response curves with depression of maximum responses: the maximum bronchoconstrictor response to histamine in the absence of FCC5 was 29 ± 4 mmHg (n = 5), and following injection of 30 μg kg^{-1} FCC5 was 15 ± 6 mmHg (n = 5) (significantly different, $P < 0.05$).

Bronchoconstrictor responses to 5-HT (4–64 μg kg^{-1}, i.v.) were attenuated by mianserin or FCC5 (Table 1). Both mianserin and FCC5 caused parallel rightward shifts of the linear portions of the log dose-response curves to 5-HT. FCC5 (10–100 μg kg^{-1}, i.v.) did not affect maximum responses to 5-HT (data not shown, n = 4–6).

The attenuation of histamine- and 5-HT-induced bronchoconstrictor responses caused by FCC5 (30–100 μg kg^{-1}, i.v.) was long lasting (> 1 h) (data not shown, n = 4). Responses to U46619 (5 μg kg^{-1}, i.v., n = 5) and acetylcholine were not affected by FCC5 (0·1–1·0 mg kg^{-1}, n = 4, data not shown). FCC5 (10–100 μg kg^{-1}, i.v.) had no effect on the histamine-induced tachycardia (data not shown, n = 4).

In a further group (n = 4–5) dose response curves to either histamine or 5-HT did not change significantly with time (i.e. when repeated after saline injection instead of the antagonists; data not shown).

5-HT-induced hindpaw oedema in the rat

FCC5 caused a dose-dependent reduction in hindpaw oedema induced by 5-HT. The ID50 values and confidence limits (95%) were 0·76 (0·47–1·16) mg kg^{-1}, i.p. and 2·7 (1·55–7·60) mg kg^{-1}, p.o. The calculated relative potency ratio for the two routes of administration was 3·55 (95% confidence limits 2·16–9·9).

Effects on the cardiovascular system

Chloralose anaesthetized rats. In chloralose anaesthetized rats, 5-HT (10–60 μg kg^{-1}, i.v.) caused triphasic responses of the arterial blood pressure. Initially, there was a fall, together with bradycardia (the Bezold-Jarisch reflex). This was followed by a pressor response and subsequently by a more prolonged depressor effect. Intravenous injection of FCC5 (0·1 mg kg^{-1}, n = 4–9) was followed by a brief rise in arterial blood pressure, together with slight tachycardia. Following this dose, the pressor response to 5-HT was abolished. After

Table 1. Antagonism dose ratios for histamine- and 5-HT-induced bronchoconstriction in guinea-pigs in the absence and presence of mepyramine, mianserin or FCC5 (n = 3–12).

Antagonist	Dose ($\mu g\ kg^{-1}$)	Antagonism dose ratio (histamine)			Antagonism dose ratio (5-HT)		
Mepyramine	1	2·56	(1·68,	4·22)		—	
	3	2·03	(1·48,	2·84)		—	
	10	26·09	(4·00,	161·44)		—	
Mianserin	3	1·94	(1·24,	3·10)	2·59	(1·56,	5·19)
	10	8·37	(4·15,	17·67)	6·64	(4·16,	11·97)
	30	206·33	(42·98,	991·92)†	26·76	(16·44,	52·99)
FCC5	3	1·21	(0·81,	1·88)		—	
	10	3·75	(2·44,	6·11)	2·03	(1·34,	3·34)
	30	11·17	(5·89,	29·28)†	4·46	(2·72,	7·61)
	100		—		18·84	(10·27,	69·11)

FCC5, mepyramine and mianserin were administered i.v. (95% confidence limits are given in parentheses.) †Significantly different from parallel (to linear portion of log dose-response curve in the absence of antagonist), $P < 0.05$.
— not measured.

FCC5 (1 mg kg^{-1}, i.v., n = 4–9), all three responses to 5-HT were either abolished or attenuated. One hour after the last dose, the Bezold-Jarisch reflex was less inhibited but other responses to 5-HT remained attenuated (data not shown, n = 4).

Pressor responses to i.v. administration of the muscarinic ganglion stimulant McNeil-A-343 (100 $\mu g\ kg^{-1}$, i.v.) were unaffected after FCC5 (1 mg kg^{-1}, n = 4; data not shown).

Pithed rats. In the pithed rat, i.v. injection of FCC5 (1 mg kg^{-1}) was followed by a long lasting slight rise in arterial blood pressure and tachycardia. Fig. 2A shows that cumulative doses of FCC5 dose-dependently inhibited pressor responses to 5-HT. Responses to 5-HT remained depressed for over an hour after injection of FCC5 (0·3 mg kg^{-1} i.v., n = 5; data not shown). Fig. 2B shows that FCC5 dose-dependently inhibited pressor responses to tyramine.

Pressor responses to stimulation of the spinal sympathetic outflow were potentiated after FCC5 (1 mg kg^{-1}, Fig. 3). Guanethidine (3 mg kg^{-1}, i.v.) abolished responses to all frequencies of nerve stimulation. However, in the presence of guanethidine and FCC5 together, responses occurred, but were less than control (Fig. 3). As shown in Table 2, FCC5 potentiated pressor responses to noradrenaline in the pithed rat. FCC5 (1 mg kg^{-1}, i.v.) had no effect on responses to methoxamine (30 $\mu g\ kg^{-1}$, i.v.) or clonidine (1 $\mu g\ kg^{-1}$) (data not shown, n = 4–5). Mianserin (0·1 mg kg^{-1}, i.v., n = 4; data not shown) inhibited pressor responses to 5-HT (10–75 $\mu g\ kg^{-1}$, i.v.) and clonidine (1 $\mu g\ kg^{-1}$, i.v., n = 4; data not shown) but unlike FCC5, had no effect on pressor responses to noradrenaline (0·3 $\mu g\ kg^{-1}$, i.v., n = 4; data not shown).

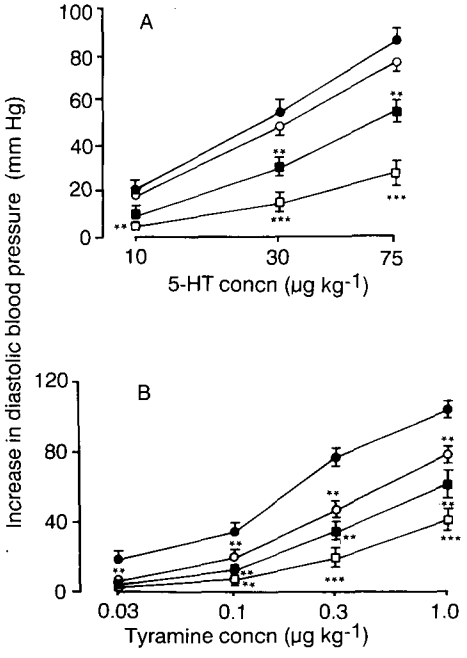

FIG. 2. Effect of FCC5 (i.v.) on the pressor responses to 5-HT (A, n = 6) and tyramine (B, n = 4–5) in the pithed rat. A. Control 5-HT pressor responses (●), responses following FCC5: 0·03 mg kg^{-1} (○), 0·1 mg kg^{-1} (■) and 0·3 mg kg^{-1} (□) are shown. B. Control tyramine pressor responses (●), responses following FCC5: 0·1 mg kg^{-1} (○), 0·3 mg kg^{-1} (■) and 1 mg kg^{-1} (□) are shown. Points are mean ± s.e. Mean response significantly different from control value. $^{**}P < 0.01$, $^{***}P < 0.001$.

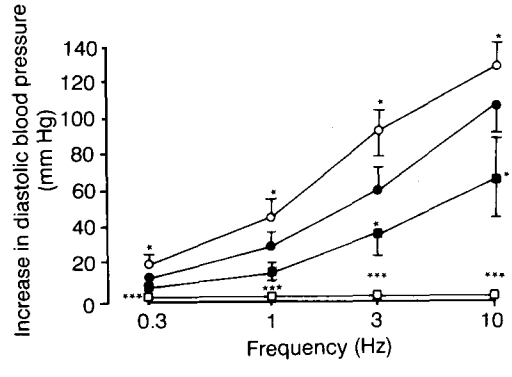

FIG. 3. Effect of FCC5 (i.v.) on pressor responses to sympathetic nerve stimulation in pithed adrenalectomized rats. Control responses (●), responses following FCC5 1 mg kg^{-1} (○); guanethidine 3 mg kg^{-1} (□); or FCC5 1 mg kg^{-1} and guanethidine 3 mg kg^{-1} (■). Points are mean ± s.e., n = 4–6. Mean response significantly different from control value. $^{*}P < 0.05$, $^{***}P < 0.001$.

Table 2. The effect of FCC5 on noradrenaline-induced increase in diastolic blood pressure in the pithed rat (n=4–5) and noradrenaline- or tyramine-induced increase in systolic blood pressure in the anaesthetized cat (n=3–5).

	Pithed rat	Anaesthetized cat	
FCC5 (mg kg^{-1})	Noradrenaline (0·3 μg kg^{-1}) ↑d.b.p. (mmHg)	Noradrenaline (0·6 μg kg^{-1}) ↑s.b.p. (mmHg)	Tyramine (60 μg kg^{-1}) ↑s.b.p. (mmHg)
0·0	47±4	41±8	47±6
0·1	62±2*	—	—
1·0	74±6**	77±21*	18±3*

FCC5, noradrenaline and tyramine were administered i.v. *P <0·05, **P <0·01; significantly different from control (i.e. no FCC5). ↑d.b.p. (mmHg)=increase in diastolic blood pressure. ↑s.b.p. (mmHg)=increase in systolic blood pressure.

Experiments using the anaesthetized cat

In chloralose-anaesthetized cats, pressor responses to tyramine were attenuated after FCC5 (Table 2) and blocked after FCC5 (3–10 mg kg^{-1}, n=2; data not shown). In one cat, which received a total dose of 10 mg kg^{-1} FCC5, pressor responses to either bilateral occlusion of the carotid arteries or injection of McNeil-A-343 (30 μg kg^{-1}, i.v.) were little affected. Pressor responses to noradrenaline were potentiated by FCC5 (Table 2). No change in magnitude of the contractions of the nictitating membrane to all frequencies of stimulation used (0·3–10 Hz) occurred after doses of FCC5 (1–3 mg kg^{-1}, n=3). Doses of 3–10 mg kg^{-1} had no obvious effect on respiratory movements or depth, but following all doses (0·1–10 mg kg^{-1}, i.v.) tachycardia and a rise in arterial blood pressure was observed (n=2; data not shown).

Actions on the CNS

Flexor reflex activity in the rat. Mianserin inhibited fenfluramine-induced facilitation of the flexor reflex in the spinal and decerebrate rat (Table 3). In contrast, FCC5 (⩽3 mg kg^{-1}, i.p.) had no significant effect (Table 3).

L-5-Hydroxytryptophan-induced head twitches in mice

The head twitch response to L-5-hydroxytryptophan in carbidopa pretreated mice was antagonized by mianserin, but not by FCC5 in doses up to 30 mg kg^{-1} i.p. (n=5) (Table 3).

Effects in conscious cats

FCC5 (3 mg kg^{-1}, s.c.) caused no behavioural changes in a cat for the following 7 h and the animal behaved normally for the next 7 days.

Table 3. ID50 values (mg kg^{-1}, i.p.) for the inhibitory actions of mianserin or FCC5 in the following CNS tests (n=5–6).

CNS test	Mianserin	FCC5
L-5-Hydroxytryptophan-induced mouse head twitch	0·11 (0·086,0·125)	>30*
Fenfluramine-facilitated rat flexor reflex activity	0·36±0·12	>3·0*

±s.e.m., or (in parentheses) 95% confidence limits. *No inhibitory effect up to the doses stated.

FCC5 (10 mg kg^{-1}, s.c.) in a second cat also caused no behavioural changes for a period of up to one week.

Two further cats were injected with FCC5 (30 mg kg^{-1}, s.c.) and coordination of movements was normal. There was no change in the tone of the nictitating membrane and pupillary and pinna reflexes were responsive.

Two of these animals subsequently received bethanidine (5 mg kg^{-1}). The nictitating membranes of both animals relaxed within 7 h and remained relaxed for the following 24 h. No other changes were noted.

Discussion

In the periphery, FCC5, like mianserin, was found to be a potent antagonist of the effects of histamine and 5-HT in-vivo in the respiratory system of the guinea-pig, and the cardiovascular systems of anaesthetized cats and anaesthetized or pithed rats. These findings parallel in-vitro observations that both FCC5 and mianserin were non-competitive antagonists of histamine and 5-HT (Leitch et al 1992).

In anaesthetized guinea-pigs, FCC5 attenuated histamine-induced bronchoconstriction with negligible effects on heart rate. Bronchoconstriction mediated by histamine in the guinea-pig lung is largely due to histamine H_1-receptor activation (Ash & Schild 1966). FCC5 (30 μg kg^{-1}, i.v.) caused a non-parallel rightward shift of the histamine log dose-response curve with reduction of the maximum response, indicating non-competitive antagonism at H_1-receptors. Mianserin, in contrast to the competitive antagonist mepyramine, caused analogous effects to FCC5.

In anaesthetized guinea-pigs, FCC5 and mianserin caused parallel rightward shifts of the log dose-response curve to bronchoconstrictor effects of 5-HT. No depression of the maximum response to 5-HT was observed after FCC5 (10–100 μg kg^{-1}, i.v.) but higher doses were not used. Airway constriction mediated by 5-HT in the guinea-pig lung is due to 5-HT_2-receptor activation (Selig et al 1988). Mianserin has previously been shown to be a competitive antagonist at 5-HT-receptors in-vitro (Doggrell 1987).

In the respiratory system of the anaesthetized guinea-pig, the inhibition of histamine and 5-HT-induced bronchoconstriction caused by FCC5 was long lasting with responses being depressed for over 1 h. Mianserin has previously been shown (Vargaftig et al 1971) to have long-lasting antihistamine activity and shorter-lasting anti-5-HT activity in the guinea-pig lung.

FCC5 showed apparent specificity for H_1- and 5-HT_2-receptors in the guinea-pig as it had no effect on bronchoconstriction caused by acetylcholine or the thromboxane A_2-mimetic, U46619.

FCC5 caused a number of effects, in-vivo, indicating that it may block the amine pump or uptake 1 mechanism responsible for termination of the effects of the noradrenergic transmitter (Iversen 1971). Intravenous administration of low doses of FCC5 was followed by sympathomimetic effects together with reduced pressor responses to the indirectly acting sympathomimetic agent tyramine and potentiation of pressor responses to both noradrenaline and sympathetic nerve stimulation in the pithed rat. In the rat, FCC5 also inhibited the inhibitory effect of guanethidine on peripheral noradrenergic nerve function. These properties are charac-

teristic of agents known to inhibit the amine pump of the noradrenergic neurone (Boura & Green 1984). The greater basicity of FCC5 relative to mianserin may be responsible for its more pronounced ability to cause amine pump blockade. The neuronal amine pump is known to be relatively nonspecific, since a large number of bases are capable of interacting with it (Boura & Green 1984).

No evidence was obtained that FCC5 depressed peripheral noradrenergic nerve function. Given acutely to pithed rats relatively large i.v. doses of FCC5 did not reduce pressor responses to sympathetic nerve stimulation. High i.v. doses did not affect contractions of the nictitating membrane of the anaesthetized cat in response to stimulation of the preganglionic cervical sympathetic nerve. Pressor responses to the muscarinic ganglionic stimulant McNeil-A-343 were also little affected after relatively large i.v. doses of FCC5 in rats and cats. These responses are readily blocked by agents decreasing noradrenergic nerve function (Boura & Green 1984). A large s.c. dose (30 mg kg^{-1}) given to conscious cats caused no relaxation of the nicititating membranes, whereas relaxation occurred in the same animals following administration of a low dose of bethanidine.

FCC5 had no effect on responses mediated by α_1- or α_2-adrenoceptors. It did not affect pressor responses to methoxamine or clonidine in the pithed rat, in the doses used. In this respect it contrasts with mianserin which shows α_2-adrenoceptor blocking properties in this species (Doxey et al 1978). In-vitro, FCC5 was also found to be devoid of α_2-adrenoceptor antagonist activity in contrast to mianserin (Leitch et al 1992).

FCC5 inhibited oedema caused by local injection of 5-HT in the hind paws of rats, an effect mediated by 5-HT_2-receptors (Peroutka 1988). The ratio p.o./i.p. dose inhibiting to an equivalent degree hind paw oedema caused by 5-HT in the rat was 3·55. Mianserin in a previous study (Vargaftig et al 1971) also showed a small ratio (approx. = 2) p.o./s.c. dose, inhibiting to an equivalent degree hind paw oedema caused by 5-HT in the rat.

The lack of effect of peripherally administered FCC5 in the two tests of CNS function provided evidence that FCC5 does not cross the blood-brain barrier. FCC5 did not depress fenfluramine-induced facilitation of the flexor reflex in rats, an effect mediated by spinal tryptaminergic mechanisms (Maj et al 1976), which we showed could be blocked by mianserin. Also, again in contrast to mianserin, FCC5 had no significant effect on L-5-hydroxytryptophan-induced head twitches in mice, an effect mediated by central 5-HT_2-receptors (Peroutka 1988).

The dissimilarities between the effects of FCC5 and mianserin can perhaps be related to their physicochemical differences. Both qualitative and quantitative differences can be attributed to the higher basicity of FCC5 due to the incorporation of the polar guanidino group attached at the 2-*N* atom of the molecule. This greater degree of basicity may be responsible not only for the changes in its peripheral effects but also for delaying its passage into the CNS, reducing central actions as compared with mianserin.

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