

Pharmacological studies with SK&F 93944 (temelastine), a novel histamine H₁-receptor antagonist with negligible ability to penetrate the central nervous system

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- 1 SK&F 93944 (temelastine), a novel histamine H₁-receptor antagonist, has been studied in a variety of *in vitro* and *in vivo* test systems.
- 2 SK&F 93944 was a competitive antagonist of histamine-induced contractions of guinea-pig ileum with a pA₂ of 9.55 and a weak, non-competitive, inhibitor of the effects of histamine on guinea-pig atrium.
- 3 In anaesthetized guinea-pigs SK&F 93944 displaced histamine bronchoconstriction dose-response curves at doses which had negligible effects on histamine tachycardia.
- 4 In anaesthetized cats SK&F 93944 antagonized depressor responses to the histamine H₁-receptor agonists, 2-(2-aminoethyl)pyridine and betahistine, at doses which had no effects on responses to the histamine H₂-receptor agonist, dimaprit.
- 5 Oral pretreatment with SK&F 93944 in conscious rats and guinea-pigs afforded protection versus the response to intradermal histamine injection.
- 6 Comparative studies in each of the test systems showed that SK&F 93944 was of comparable or significantly greater potency than the standard compound, mepyramine.
- 7 SK&F 93944 was found to be a weak, non-competitive antagonist of carbachol on the guinea-pig ileum but was devoid of measurable anticholinergic activity *in vivo*.
- 8 Studies on the penetration of [¹⁴C]-SK&F 93944, labelled either in the isocytosine ring or in the butyl chain, showed that brain concentrations were very low when compared with the steady-state blood concentrations. In contrast, brain concentrations of [³H]-mepyramine exceeded blood concentrations by a factor of approximately 3.
- 9 SK&F 93944 may have an advantage over classical histamine H₁-receptor antagonists in that it is likely to be devoid of untoward effects on the central nervous system.

Introduction

Histamine antagonists are widely used in the treatment of a range of allergic disorders, in particular, allergic rhinitis. Use of these agents has been limited by side-effects, usually sedation, associated with the action of these drugs within the central nervous system (CNS). This has prompted research directed towards the discovery and development of histamine H₁-receptor antagonists with a reduced liability to cause CNS side-effects. A number of such compounds have been

described including azatadine (Tozzi *et al.*, 1974), astemizole (Van Wauwe *et al.*, 1981) terfenadine (Cheng & Woodward, 1982) and mequitazine (Le Fur *et al.*, 1981).

SK&F 93944 (temelastine), 2-[4-(3-methyl-5-bromopyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)methyl]-4-pyrimidone, is a novel histamine H₁-receptor antagonist (Figure 1). This compound was developed from the combined histamine H₁/H₂-receptor antagonist, SK&F 93319 (Blakemore *et al.*, 1983; Harvey & Owen, 1984) and differs from previously

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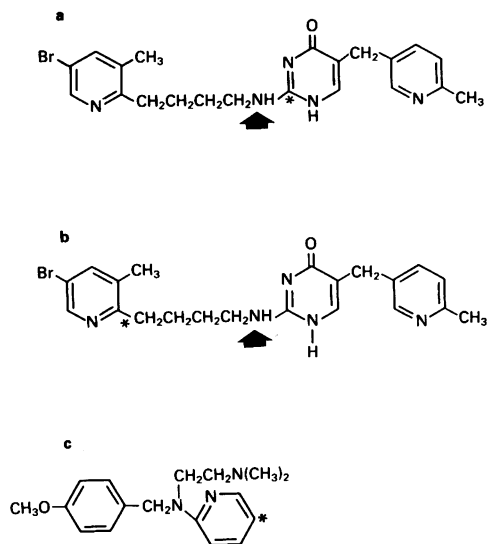


Figure 1 Structures of both SK&F 93944 (a and b) and mepyramine (c). The asterisks indicate the position of the radiolabels used in the CNS penetration studies; two radiolabelled compounds were prepared and used for studies with SK&F 93944. The arrows indicate the position of hydrolytic cleavage *in vivo*.

described H_1 -receptor antagonists in that it lacks a tertiary amino group and is largely unprotonated at physiological pH.

This paper describes the pharmacology of SK&F 93944 in a range of *in vitro* and *in vivo* models designed to evaluate both its potency and selectivity. In addition, studies are described which show that the compound has negligible ability to cross the blood-brain barrier in rats, and is consequently highly unlikely to elicit CNS side-effects. A preliminary account of this work was presented at the Birmingham meeting of the British Pharmacological Society, April 1984 (Durant *et al.*, 1984a).

Methods

In vitro studies

Tissues were obtained from freshly killed Dunkin-Hartley guinea-pigs of either sex, weighing 400–700 g.

Preparations of terminal ileum were mounted in 10 ml baths containing magnesium-free Tyrode solution at 30°C and gassed with 5% CO_2 and 95% O_2 . A load of 0.5 g was applied to the tissue.

Atrial strips, including the sino-atrial node, were suspended in 15 ml baths in McEwan's solution at 34°C, gassed with 5% CO_2 and 95% O_2 . The muscle was loaded to 300 mg tension.

Cumulative agonist dose-response curves were obtained prior to and following incubation with a range of antagonist concentrations. Incubation times of 8 and 60 min were used for the ileum and atrium respectively as preliminary experiments indicated no greater antagonism at longer equilibration times. Where appropriate, antagonist potency was expressed in terms of pA_2 values.

In vivo studies

Studies in anaesthetized guinea-pigs Male Dunkin-Hartley guinea-pigs, 350–450 g, were anaesthetized by an intraperitoneal (i.p.) injection of sodium pentobarbitone, 60 mg kg^{-1} . Blood pressure was recorded from a cannula inserted into one carotid artery and the pulse pressure used to trigger an instantaneous rate meter to measure heart rate. The jugular vein was cannulated for administration of drugs and supplementary anaesthetic.

Animals were artificially ventilated through a tracheal cannula using the volume of air just required to fill the lungs at an airway pressure of 12 cmH_2O . A pressure transducer was inserted into the outflow limb of the system to measure airway inflation pressure as an index of resistance. Blood pressure, heart rate and airway pressure were registered continuously on an electronic recorder (Lectromed, M19).

Dose-response curves for histamine-induced bronchoconstriction and tachycardia were constructed in each animal prior to and following intravenous injection of a histamine H_1 -receptor antagonist at a series of doses. In a further series of experiments with SK&F 93944, carbachol was used as the agonist to elicit bronchoconstriction.

Studies in anaesthetized cats Studies were carried out in cats of either sex, body weight 1.65–3.15 kg. Following induction of anaesthesia the trachea was cannulated. Systemic blood pressure was measured from a catheter in one carotid or femoral artery and monitored on a Lectromed M19 recorder. Heart rate was measured with an instantaneous rate meter triggered by the blood pressure pulse. Injections of drugs and supplementary doses of anaesthetic were made via cannulae inserted into the femoral and/or brachial veins.

Studies with selective histamine receptor agonists Cats were anaesthetized with sodium pentobarbitone, 60 mg kg^{-1} i.p. Responses to 2-(2-aminoethyl) pyridine (2-PEA), betahistine and dimaprit were obtained in separate groups of cats, using procedures described by Owen (1975). Dose-response curves for depressor responses, quantified as percentage falls in diastolic blood pressure, to each agonist were constructed before and following intravenous injection of

at least two antagonist doses.

Analysis of variance was used to estimate the displacement of agonist dose-response curves by comparison of the potency of the agonist in the presence and absence of antagonist. The analysis was restricted to a 2 + 2 dose comparison over the range where response was linear with respect to log dose; a test for parallelism was incorporated into the programme.

Studies on cutaneous permeability

Rats Studies were carried out in male, Wistar rats 200–250 g; the skin covering the dorsal trunk was shaved closely the day before the experiment. Animals were starved for approximately 18 h but allowed free access to water.

Intradermal injections of histamine, 20, 50 and 100 µg, were made under light halothane (ICI)/oxygen anaesthesia and randomly sited over the shaved skin. The injection volume was 50 µl and each animal additionally received an injection of the vehicle alone. Five minutes before the histamine injection each rat was given 1 ml 0.25% Evans Blue solution, containing 500 nCi [¹²⁵I]-human serum albumin (HSA, Radiochemical Centre), via a tail vein. Rats received either antagonist or vehicle intravenously or orally (2 ml kg⁻¹) at appropriate times prior to histamine challenge.

Vascular permeability changes were measured 15 min after injection of histamine since preliminary experiments indicated this time to be optimal in the rat. At this time the rats were again anaesthetized, the abdomen opened and a 1 ml sample of blood taken from the vena cava. The animals were then killed and the blued skin sites punched out with a cork borer (ca. 19 mm diameter).

Blood and skin samples were counted for ¹²⁵I content in a Packard Autogamma Scintillation Spectrometer (No. 5221); skin samples were weighed after oven drying to constant weight.

Vascular permeability was calculated as

$$\frac{\text{skin count}}{\text{blood count} \times \text{weight}}$$

and expressed as ml blood g⁻¹ dry weight. The net response to histamine was calculated by subtraction of the response to the vehicle alone.

Guinea-pigs Studies were made in Dunkin-Hartley guinea-pigs of either sex, 400–500 g, using a similar procedure to that adopted in the conscious rats and prepared as described above. Intradermal injections of histamine, 1, 10 and 100 µg were made under light

ether anaesthesia using an injection volume of 10 µl; vascular permeability changes were measured 30 min later. Identification of injection sites and quantification of the permeability response was achieved by injecting 0.2 ml of a 2.5% solution of Evans Blue containing 250 nCi [¹²⁵I]-HSA via a dorsal foot vein. Antagonists and vehicle were given orally using a dose-volume of 1 ml kg⁻¹.

In studies on both rats and guinea-pigs inhibition of histamine responses by an antagonist was expressed as the percentage reduction of the net response to 100 µg histamine in treated animals compared to vehicle-treated controls. The response to 100 µg histamine represents a large but submaximal response in both species (e.g. Owen *et al.*, 1984).

Studies on autonomic nervous system function and haemodynamics Cats were anaesthetized with an intraperitoneal injection of α-chloralose (80 mg kg⁻¹), mixed with sodium pentobarbitone (6 mg per cat). The trachea was cannulated.

Parasympathetic nervous system The cervical vagi were sectioned and the peripheral end of the left nerve stimulated at 10 min intervals prior to, during and following 30 min intravenous infusions of SK&F 93944. Stimulation parameters were: strength 4 V, frequency 2 Hz, pulse width 4 ms, duration 30 s. Responses were quantified as maximum evoked bradycardia.

Sympathetic nervous system Pressor responses and tachycardia elicited during bilateral carotid occlusion and by intravenous injection of noradrenaline, 0.1 µg kg⁻¹, were measured before, during and for 1 h after administration of SK&F 93944.

Studies on haemodynamic function The animals were maintained on artificial respiration. Blood pressure, from a cannula in one femoral artery, heart rate, from the blood pressure pulse, and aortic blood flow, using an electromagnetic flow probe were measured before, during and for 1 h after administration of SK&F 93944. In each of these studies, SK&F 93944 was administered by intravenous infusion over 30 min at total doses of 0.1, 1 and 10 mg kg⁻¹.

Penetration of [¹⁴C]-SK&F 93944 and [³H]-mepyramine into the brain in anaesthetized male rats Male rats, body weight ~ 250 g, were starved overnight and then anaesthetized by intraperitoneal injection of a urethane solution (25% w/v, 1.5 g kg⁻¹). The trachea and one femoral vein were cannulated. An intravenous (i.v.) bolus (2 ml) followed by an i.v. infusion (rate 2.0 ml h⁻¹ for 2 to 3.75 h) of the dose solution were administered to each rat via the femoral vein. The infusion was maintained until the total

radioactivity in the blood achieved a plateau, or until all the available dose had been administered. Duplicate blood samples (10 μ l) were taken at intervals from a lateral tail vein and haemolysed in distilled water (0.5 ml) before liquid scintillation counting, in order to monitor the radioactivity in the blood. At the end of the infusion, the rat was exsanguinated and the brain was removed. It was rinsed in chilled saline and then dissected on a refrigerated glass plate into 11 regions. These were weighed and then dissolved using Soluene-100 (Packard Instrument Ltd.) (1 ml for 150 mg tissue, 40°C, overnight). Glacial acetic acid (50 μ l per ml Soluene) was then added to each sample, to prevent chemiluminescence, and the samples were assayed for radioactivity by liquid scintillation counting.

Measurement of blood volume in the rat brain In order to correct the concentration of parent, or parent-related, material in the brain for radioactivity present in the residual blood in the brain, it was necessary to measure the volume of this blood under the present experimental conditions. [3 H]-inulin (Amersham International plc) was originally included in the [14 C]-SK&F 93944 dose solution, as a blood marker, in order to make a direct correction. It became apparent, however, that [3 H]-inulin was an inadequate blood marker in these studies, since some 3 H material was entering the brain tissue. The residual blood in the brain was therefore measured, using [14 C]-methylhydroxyinulin (Amersham International plc) or 51 Cr-labelled rat erythrocytes (Ebaugh *et al.*, 1953) as blood markers, following dosing with unlabelled SK&F 93944 under the same conditions as in the brain penetration studies. [14 C]-methylhydroxyinulin was included in the dose solutions of [3 H]-mepyramine as a direct blood marker.

Drugs used

Drugs used were:- SK&F 93944 (temelastine); 2-(2-aminoethyl)pyridine dihydrochloride (2-PEA), dimaprit dihydrochloride, betahistine dihydrochloride (all Smith Kline & French Research Ltd), histamine acid phosphate (B.D.H.).

NaCl 0.9% w/v (saline) was used as the vehicle for all *in vivo* studies. SK&F 93944 was dissolved in a small volume of 1N HCl, the pH adjusted to about 6, and the solution made up to volume with saline.

[14 C]-SK&F 93944 free base labelled with 14 C at two alternative positions, was prepared by the SK&F Department of Synthetic and Isotope Chemistry. The two alternative positions of the radiolabel are shown in Figure 1. The specific activity of the isocytosine labelled material was 13.45 Ci mol $^{-1}$; the specific activity of the butyl chain labelled material was 57.5 Ci mol $^{-1}$.

In the brain penetration studies, [14 C]- and unlabelled SK&F 93944 were dissolved in a minimal quantity of saline and hydrochloric acid. The pH was adjusted to between 4 and 7 with sodium hydroxide solution prior to making up to volume with saline. The dose solutions were analysed by thin layer chromatography and the radiochemical purities were quantified using a linear analyser (Berthold, LB2832). The radiochemical purity of the dose solutions was at least 97% in all studies.

Results

In vitro studies

SK&F 93944, equilibration time 8 min, produced concentration-dependent inhibition of histamine-induced contractions of the guinea-pig ileum over the concentration range, 2.2×10^{-10} – 2×10^{-9} M, pA_2 9.55 (9.38–9.82, 95% confidence limits) and the regression of the Schild plot, 0.89 (0.63–1.15) was not significantly different from unity. The antagonism produced by SK&F 93944 was readily reversible; a 16% depression of the maximum response to histamine was recorded at a concentration of 2×10^{-9} M. In comparative experiments with mepyramine a pA_2 of 8.89 (8.77–9) was calculated with 34% and 66% depressions of maxima at concentrations of 2×10^{-9} and 5×10^{-9} M, respectively.

SK&F 93944 (2×10^{-6} – 5.4×10^{-5} M) was a weak, non-competitive antagonist at H_2 -receptors, inhibitor of histamine-induced tachycardia on the guinea-pig atrium and a very weak, non-competitive inhibitor of carbachol on the guinea-pig ileum, dose-ratio (DR) = 2 at 1.07×10^{-4} M. No inhibition of isoprenaline-induced tachycardia on guinea-pig atrium was measured at concentrations of SK&F 93944 up to 4.86×10^{-4} M; at these high concentrations SK&F 93944 produced marked negative chronotropic effects.

In vivo studies

Effects on histamine-induced bronchoconstriction and tachycardia in anaesthetized guinea-pigs In untreated animals histamine evoked dose-related bronchoconstriction and tachycardia over the dose range 2×10^{-8} to 3.2×10^{-7} mol kg $^{-1}$.

The dose-response curve for histamine-induced increases in airway pressure was shifted progressively to the right by SK&F 93944, 1.25×10^{-9} – 7.5×10^{-8} mol kg $^{-1}$.

It was not possible to make a true potency comparison between SK&F 93944 and mepyramine in this test system since the regression of log (DR – 1) upon log (dose) for the two antagonists were not parallel. However, reference to the doses required to give

Table 1 Antagonism of histamine-induced bronchoconstriction and tachycardia by SK&F 93944 in anaesthetized guinea-pigs

	Antagonist, dose (mol kg ⁻¹)	Bronchoconstriction	Tachycardia
SK&F 93944	1.25 × 10 ⁻⁹	1.32 ± 0.13 (6)	1.27 ± 0.29 (5)
	1.25 × 10 ⁻⁸	1.96 ± 0.29 (8)	1.79 ± 0.70 (6)
	3.75 × 10 ⁻⁸	16.1 ± 5.39 (8)	2.28 ± 0.64 (5)
	7.5 × 10 ⁻⁸	51.8 ± 11.2 (4)	3.12 ± 0.22 (4)
Mepyramine	2.5 × 10 ⁻⁹	2.64 ± 0.38 (4)	—
	1.25 × 10 ⁻⁸	5.21 ± 1.15 (4)	—
	1.25 × 10 ⁻⁷	50.7 ± 12.6 (5)	—

Results show mean dose-ratios ± s.e.mean of *n* (number in parentheses) experiments.

(DR - 1) = 10 for each antagonist, 3 × 10⁻⁸ mol kg⁻¹ and 2.8 × 10⁻⁸ mol kg⁻¹ for SK&F 93944 and mepyramine respectively, indicated that the two compounds were essentially equipotent.

In contrast to its effects on bronchoconstriction, SK&F 93944 had little inhibitory action on histamine-evoked tachycardia and produced only modest displacements of the dose-response curves (Table 1). A dose of 7.5 × 10⁻⁸ mol kg⁻¹, which produced a greater than 50 fold shift of the bronchoconstriction dose-response curve, displaced the tachycardia response with a dose-ratio of 3.12 ± 0.22 (mean ± s.e., *n* = 4).

Effects on depressor responses to H₁- and H₂-receptor agonists in anaesthetized cats SK&F 93944, 2.3 × 10⁻⁷ and 1.13 × 10⁻⁶ mol kg⁻¹ i.v., produced a dose-related inhibition of responses to the H₁-receptor agonist 2-PEA with parallel dextrad displacements of the agonist dose-response curve (Figure 2); calculated

dose-ratios were 11.9 (7.46–19.2, 95% confidence limits, *n* = 4) and 62.5 (40–111, *n* = 4) respectively. Responses to 2-PEA were additionally inhibited by mepyramine, 2.5 × 10⁻⁶ and 2.5 × 10⁻⁵ mol kg⁻¹ i.v., resulting in dose-ratios of 7.4 (2.69–20.6, *n* = 4) and 54.9 (19.7–152, *n* = 4) respectively. From the regression of log (DR - 1) upon log dose, SK&F 93944 was calculated to be between 19 and 24 times more potent than mepyramine.

SK&F 93944 was similarly effective at antagonizing responses to a second H₁-receptor agonist, betahistine, producing very similar displacements of this agonist dose-response curve, at any given dose, to those seen with 2-PEA (Figure 2).

In contrast, SK&F 93944 1.13 × 10⁻⁶ mol kg⁻¹ failed to produce any shift of the dose-response curve to the H₂-receptor agonist, dimaprit; the calculated dose-ratio was 1.0 (0.8–1.2, *n* = 4).

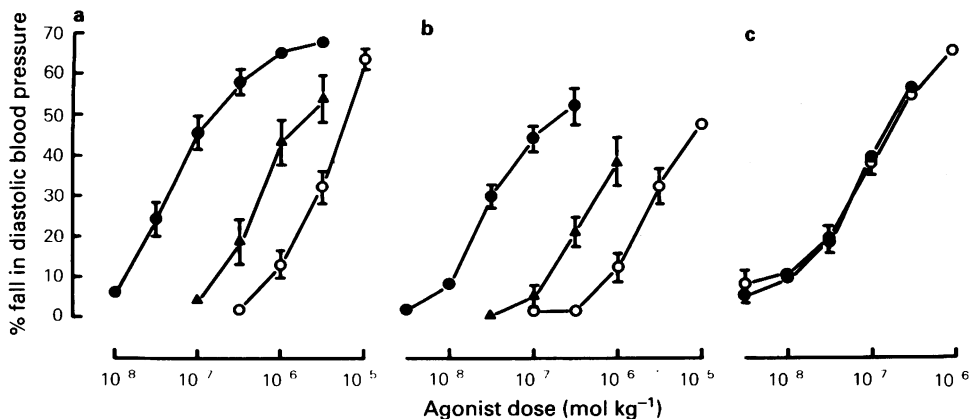


Figure 2 Antagonism of depressor responses to 2-(2-aminoethyl) pyridine (a), betahistine (b) and dimaprit (c) by SK&F 93944. Control agonist dose-response curve (●), responses following administration of SK&F 93944, 2.3 × 10⁻⁷ mol kg⁻¹ (▲) and 1.13 × 10⁻⁶ mol kg⁻¹ (■), are shown. Points are mean and vertical lines s.e., *n* = 4.

Antagonism of histamine-induced increases in cutaneous microvascular permeability in rats and guinea-pigs Histamine elicited dose-dependent increases in microvascular permeability as measured by the net accumulation of [125 I]-HSA at the sites of histamine intradermal injection in both rats and guinea-pigs. Intravenous pretreatment with SK&F 93944, 1 and 5 mg kg $^{-1}$, in the rat resulted in dose-related inhibition of the histamine response; with a histamine challenge dose of 100 μ g, the protection afforded was 60.3% and 94.1% respectively (Figure 3). Mepyramine, 5 mg kg $^{-1}$, produced a 91% inhibition of the same response, indicating similar potencies of the two antagonists after intravenous administration in this test system.

Oral pretreatment of both rats and guinea-pigs with SK&F 93944 or mepyramine also inhibited the response to intradermal histamine in a dose-dependent manner. In both species, using this route of administration, SK&F 93944 was clearly more potent than mepyramine (Figures 3 and 4). SK&F 93944 produced dose-related inhibition over the dose range 1–15 mg kg $^{-1}$ in the rat and 0.001–0.5 mg kg $^{-1}$ in the guinea-pig. In corresponding studies mepyramine was effective at doses of 10–40 mg kg $^{-1}$ and 0.004–2.5 mg kg $^{-1}$ respectively.

In further studies in the rat the oral dose of SK&F 93944 was fixed at 5 mg kg $^{-1}$ and the pretreatment time varied between 15 and 120 min to give some indication of the onset and duration of action of the compound. The largest inhibition (86%) occurred with the earliest pretreatment time of 15 min; inhibition declined progressively with longer pretreatment times (Figure 5).

The time-course of the response to SK&F 93944 was determined using two oral doses in the guinea-pig – 0.004 mg kg $^{-1}$ which produced sub-maximal inhibition (Figure 4) and 0.1 mg kg $^{-1}$ which elicited a near-maximal effect. In each case maximal inhibition, 63% and 87% respectively, was measured after 60 min; inhibition was not significantly reduced after 120 min.

Antimuscarinic effects Carbachol administration in anaesthetized guinea-pigs elicited dose-dependent bronchoconstriction over the dose range 1×10^{-8} – 8×10^{-8} mol kg $^{-1}$. At doses of 1.25×10^{-8} – 7.5×10^{-8} mol kg $^{-1}$, which markedly antagonized histamine-induced bronchoconstriction, SK&F 93944 failed to displace significantly the corresponding dose-response curve for carbachol.

Effects on autonomic nervous system function The bradycardia elicited in anaesthetized cats by vagal stimulation was not significantly altered during or following infusions of SK&F 93944 0.1, 1 or 10 mg kg $^{-1}$. In addition, SK&F 93944 had no effect on the pressor responses and tachycardia elicited by

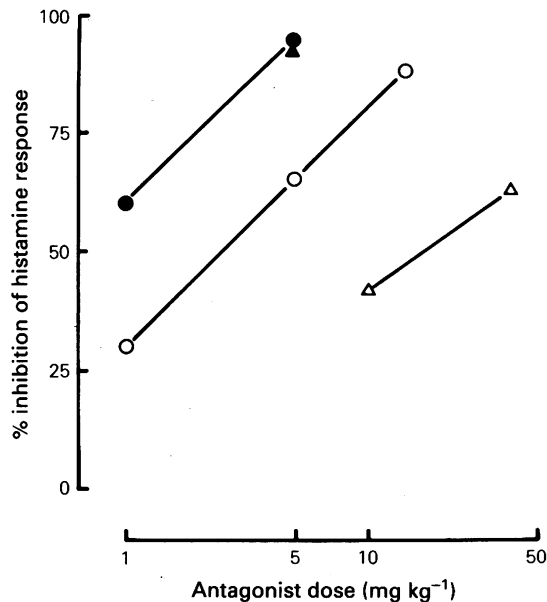


Figure 3 Inhibition of the increase in vascular permeability produced by 100 μ g histamine i.d. in the rat by oral (open symbols) and intravenous (filled symbols) pretreatment with SK&F 93944 (●,○) and mepyramine (▲,△). Sixty minutes and 15 min pre-dosing times were used in oral and intravenous studies, respectively.

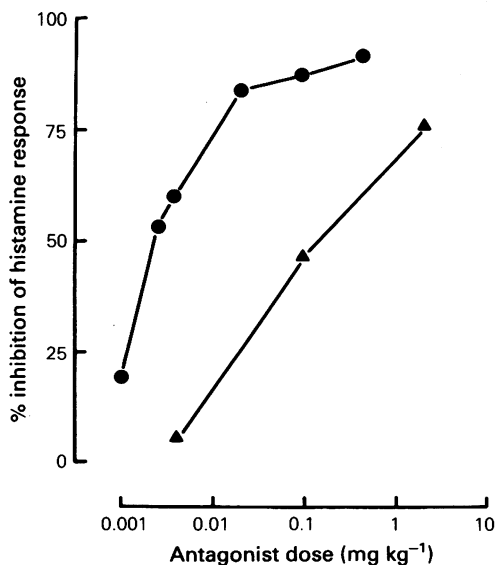


Figure 4 Inhibition of the increase in vascular permeability produced by 100 μ g histamine i.d. in the guinea-pig by oral pretreatment with SK&F 93944 (●) and mepyramine (▲).

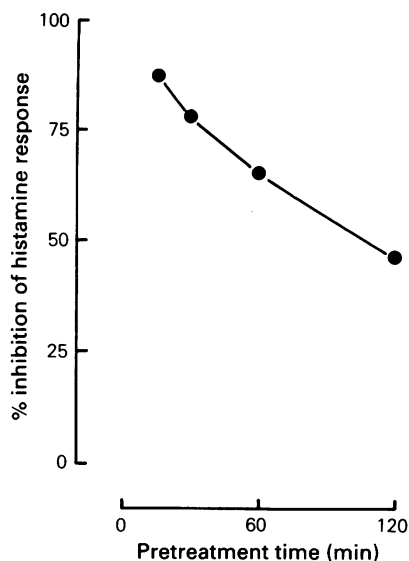


Figure 5 Time course of the inhibition of histamine-induced increases in vascular permeability, in rats. SK&F 93944 was administered at 5 mg kg⁻¹.

bilateral carotid occlusion or intravenous injection of noradrenaline, 0.1 µg kg⁻¹, over the dose range 0.1–10 mg kg⁻¹.

Effects on haemodynamic function SK&F 93944, 0.1–10 mg kg⁻¹ had no effect on blood pressure, heart rate or aortic blood flow in anaesthetized cats.

Blood volume in the rat brain The residual blood volume in the brain, measured using [³H]-inulin, was considerably larger than that measured using [¹⁴C]-methylhydroxyinulin or ⁵¹Cr-labelled erythrocytes (Table 2). The values obtained using these latter two blood markers (12 or 15 µl blood per g brain tissue) were also close to the literature values (Altman & Dittmer, 1961). A value of 15 µl per g was therefore used to correct the brain concentrations of total radioactivity for any radioactivity present in the residual blood in the studies using [¹⁴C]-SK&F 93944. Although [¹⁴C]-methylhydroxyinulin proved to be an adequate blood marker when included in the dose solution in the studies using [³H]-mepyramine, the value of 15 µl blood g⁻¹ brain tissue was again used to correct for residual blood, in order to allow direct comparisons.

Penetration of [¹⁴C]-SK&F 93944 and [³H]-mepyramine into the brain in anaesthetized male rats The blood concentrations of total ¹⁴C radioactivity at the end of the infusions of [¹⁴C]-SK&F 93944 ranged

Table 2 Residual blood volumes in the rat brain, as measured by [¹⁴C]-methylhydroxyinulin, ⁵¹Cr-labelled rat erythrocytes and [³H]-inulin, in the presence of unlabelled SK&F 93944

Blood marker	Blood volume (µl g ⁻¹ brain tissue)
[¹⁴ C]-methylhydroxyinulin	15 ± 2
⁵¹ Cr-labelled rat erythrocytes	12 ± 2
[³ H]-inulin	63 ± 14

Results show mean values ± s.d.

from 2.7 × 10⁻⁶ to 3.19 × 10⁻⁴M which is equivalent to 2.7 × 10⁻⁹ to 3.03 × 10⁻⁷ mol g⁻¹, assuming a specific gravity of 1.054 for rat blood (Altman & Dittmer, 1961). It was not, however, always possible to achieve steady state.

The distribution of ¹⁴C radioactivity in each brain studied was homogeneous and the brain concentrations shown in Figure 6 are means of eleven regions of each brain.

The mean brain: blood concentration ratio (defined as mol g⁻¹ in brain: mol g⁻¹ in blood) ± s.d. (corrected for residual blood) following dosing with [¹⁴C]-SK&F 93944 labelled at position C-2 of the isocytosine ring was 0.012 ± 0.004 (12 rats) and 0.015 ± 0.004 (7 rats) following dosing with [¹⁴C]-SK&F 93944 labelled at position C-4 of the butyl chain.

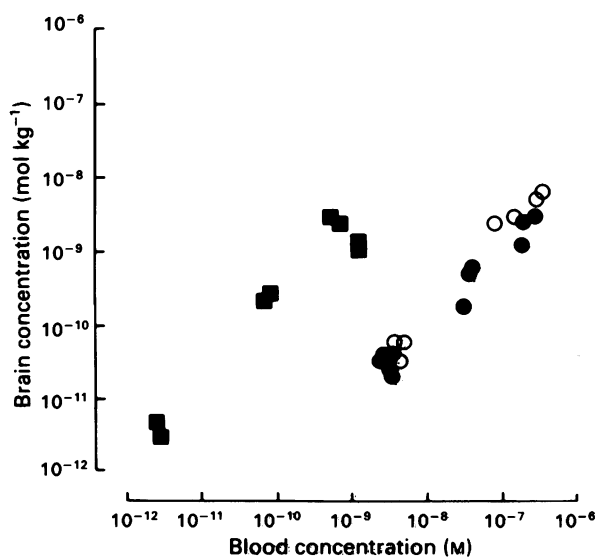


Figure 6 Blood concentration plotted against brain concentration in anaesthetized rats. Values shown are individual values for mepyramine (■), and SK&F 93944, isocytosine label (●) and butyl chain label (○).

The blood concentrations of total ^3H radioactivity at the end of the infusions of [^3H]-mepyramine ranged from 2.4×10^{-9} to $2.38 \times 10^{-6}\text{M}$ (which is equivalent to 2.3×10^{-12} to $2.26 \times 10^{-9}\text{mol g}^{-1}$). The distribution of radioactivity in these studies was also homogeneous and the brain concentrations shown in Figure 6 are means of the eleven regions of each brain. The mean brain:blood concentration ratio \pm s.d. (corrected for residual blood in the brain) following dosing with [^3H]-mepyramine was 3.16 ± 1.48 .

The relationship between blood concentrations and brain concentrations for each rat dosed with [^{14}C]-SK&F 93944 or [^3H]-mepyramine are illustrated in Figure 6.

Discussion

This paper describes the results of pharmacological studies with SK&F 93944 which demonstrate that the compound is a potent, selective histamine H_1 -receptor antagonist both *in vitro* and *in vivo*. SK&F 93944 proved to be of equivalent or significantly greater potency to mepyramine, the reference compound included in each of the studies.

SK&F 93944 was derived from SK&F 93319, a combined histamine H_1 - and H_2 -receptor antagonist with similar pA_2 s on the two receptor systems (Blake-more *et al.*, 1983), by procedures designed to optimize H_1 - and minimize H_2 -receptor antagonism whilst retaining lack of CNS penetration (Durant *et al.*, 1984b). SK&F 93944 was a highly potent H_1 -antagonist (pA_2 9.55) showing competitive inhibition up to concentrations of $1.34 \times 10^{-9}\text{M}$ at and above which some depression of histamine maximum occurred. *In vitro* studies showed that SK&F 93944 inhibited the effects of histamine on guinea-pig atrium at concentrations some 5000 times those required to antagonize the effects of this agonist on guinea-pig ileum indicating a marked receptor selectivity. The selectivity of SK&F 93944 for H_1 - versus H_2 -receptors was also evident in *in vivo* models designed to separate these two antagonistic actions.

The anaesthetized guinea-pig model was developed specifically to permit simultaneous measurement of histamine H_1 - and H_2 -receptor antagonists *in vivo*. Histamine elicits dose-dependent bronchoconstriction and tachycardia in this preparation via interaction with H_1 - and H_2 -receptors respectively; each response may be selectively inhibited with appropriate antagonists (Owen & Pipkin, 1985).

SK&F 93944 produced parallel, dose-related dextrad displacements of the histamine bronchoconstriction curve with negligible effects on heart rate, consistent with *in vivo* selectivity for H_1 - versus H_2 -receptors.

In vivo selectivity was further confirmed in anaesthetized

cats utilizing, as agonist responses, the falls in blood pressure elicited by betahistine, 2-PEA, and dimaprit. SK&F 93944 showed similar activity against both of the H_1 -agonists, but at doses producing a greater than 60 fold shift of these dose-response curves failed to produce any displacement of the dimaprit response (dose-ratio 1.0).

Both mepyramine and SK&F 93944 were effective antagonists of histamine-induced increases in vascular permeability in rats and guinea-pigs. However, the two compounds were found to differ markedly with respect to oral potency, SK&F 93944 demonstrating significantly greater oral activity than mepyramine.

The selectivity of SK&F 93944 as an H_1 -receptor antagonist was further demonstrated by its lack of antimuscarinic activity on guinea-pig ileum, guinea-pig respiratory smooth muscle and bradycardia due to vagal stimulation in anaesthetized cats. Further, SK&F 93944 had no detectable effect on other facets of autonomic nervous system function or on haemodynamics in anaesthetized cats when administered at doses in excess of those needed to elicit pronounced H_1 -receptor antagonism.

The brain penetration studies provided a measure of the capacity of radiolabelled SK&F 93944 or mepyramine (and those labelled metabolites which are present in the systemic circulation) to enter the brain at given steady state blood concentrations. The method of drug administration used in these studies was designed to achieve steady state blood concentrations of total radioactivity as soon as possible. At steady state, radiolabelled drug in the blood would be expected to be in equilibrium with the major tissues of the body, which could include the brain. Even if equilibrium is not established between blood and brain, this procedure allows the maximum practical opportunity to approach such a state and is preferable to other methods of dosing.

In these studies, [^3H]-mepyramine and related material were shown to be present in the brain at a similar or somewhat greater concentration than in the blood at steady state (300% of that in the blood). In contrast, the concentrations of [^{14}C]-SK&F 93944 and ^{14}C -metabolites achieved in the brain were minimal (1–2%) when compared with those in the blood.

In the absence of any other consideration, at true equilibrium the concentration of free drug ought to be the same on either side of the blood-brain barrier. An uncharged lipophilic compound such as SK&F 93944 based on a log P value of approx. 3.57 at pH 7.4, determined by calculation from a measured log P (octanol/borax-boric acid buffer) of 3.59 at pH 8.4 (C.J. Salter, personal communication) would be expected to equilibrate with the brain relatively rapidly, and hence, under the steady state conditions of these experiments, it is arguable that a good approximation of equilibrium would be achieved. A principal factor

mediating against equal concentrations of SK&F 93944 being established on either side of the barrier would therefore be expected to be the effects of protein binding. The relative affinity for binding sites in the blood and in the brain would determine to a large extent the distribution of total drug. The proportion of SK&F 93944 bound in rat serum, as measured by equilibrium dialysis *in vitro*, is approximately 99.3–99.1% over the concentration range 0.14–100 μM (E.A. Brown, unpublished results), so that the free concentration of the compound is small. The low brain concentration observed in these experiments might then be argued to reflect a much greater affinity of SK&F 93944 for serum proteins than brain tissue as a whole. However, in view of the lipophilic character of the compound it is considered unlikely that the binding of brain tissue is insignificant. The comparative study with mepyramine (log P octanol/Na/K phosphate buffer 1.9 at pH 7.4) demonstrates that this compound certainly does bind to brain tissue, as at steady state it is maintained in the brain against an apparent concentration gradient (when total drug is measured).

The blood concentrations established (up to $3.19 \times 10^{-4}\text{M}$) covered a range which extended far in excess of those which would have been reached with the doses used in the pharmacological studies. Similar doses to the largest used in pharmacological testing in the rat have been shown to produce blood concentrations of SK&F 93944 of approximately $1 \times 10^{-5}\text{M}$, (5 min after an i.v. bolus injection of 3 mg kg⁻¹ and at peak concentration after 30 mg kg⁻¹ p.o.). The potential of SK&F 93944 to produce significant central effects at doses which are pharmacologically active in peripheral systems is therefore small.

In these studies measurement of radiolabelled material in the brain tissue necessarily includes radioactive compound which is present in the residual blood in the brain. Precise calculation of the amount of compound in this residual blood is less important for drugs which readily penetrate the CNS e.g. mepyramine, because the amount of compound in the blood will be small compared to the amount present in the brain tissue itself. However, the measure of the CNS penetration of a compound such as SK&F 93944 which exhibits minimal distribution into the brain

requires an accurate knowledge of the residual blood volume in the brain (see Table 2), because this blood contains almost the entire drug associated with the whole brain.

The metabolism of SK&F 93944 in the rat involves a hydrolytic cleavage of the molecule at the position indicated in Figure 1. Hence, the two alternative positions of the radiolabel result in apparently different metabolic patterns (Cox *et al.*, 1984). However, the two alternative radiolabelled forms of [¹⁴C]-SK&F 93944 gave similarly low brain: blood concentration ratios, suggesting that neither parent compound nor the ¹⁴C-metabolites from the two alternative forms penetrated the rat brain to any significant extent. At a given steady state blood concentration, therefore, the brain penetration of [¹⁴C]-SK&F 93944 in the anaesthetized male rat was at least 200 times less than that of [³H]-mepyramine.

SK&F 93944 is of particular interest as a potent, selective histamine H₁-receptor antagonist because these properties are coupled with negligible penetration of the compound into the brain.

In addition to sedation and other CNS effects, a significant disadvantage to established antihistamines is their anticholinergic side-effects resulting in, for example, dry mouth. SK&F 93944 showed some non-competitive antimuscarinic activity on guinea-pig ileum but was some 400,000 times less active in this respect than against the effects of histamine on the same tissue. In two *in vivo* preparations, at concentrations known to produce marked histamine H₁-receptor antagonism, SK&F 93944 showed no measureable anticholinergic activity.

In conclusion, SK&F 93944 is a novel, potent, selective and orally-active antihistamine which is predicted to be devoid of antimuscarinic and sedative side-effects. Clinical pharmacology studies, including an assessment in healthy volunteers (Boyce, 1984), are ongoing to confirm the profile of this compound in man and to evaluate its efficacy in a range of allergic disorders.

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