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Zolantidine (SK&F 95282) is a potent selective brain-penetrating histamine H_2-receptor antagonist

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1 The novel benzthiazole derivative zolantidine (SK&F 95282) is a potent antagonist of histamine at H_2-receptors in guinea-pig atrium and rat uterus. Only apparent pA_2 values of 7.46 and 7.26 respectively could be calculated since the slopes of the Schild plots were significantly less than unity.

2 Zolantidine is equally potent as an antagonist at histamine H_2-receptors in guinea-pig brain. The compound inhibited histamine stimulated adenylate cyclase (pK_i 7.3) and dimaprit stimulated adenosine 3':5'-cyclic monophosphate (cyclic AMP) accumulation (approx pA_2 7.63), and competed with [^{3}H]-tiotidine binding (pK_i 7.17).

3 Zolantidine is at least 30 fold more potent at H_2-receptors than at other peripheral and central receptors investigated.

4 Infusion of zolantidine into rats produces a brain concentration greater than the plateau blood concentration (brain/blood ratio 1.45).

5 Zolantidine is thus characterized as a potent selective brain-penetrating H_2-receptor antagonist, and will be a valuable pharmacological tool for investigating possible physiological and pathological roles for histamine in the central nervous system.

Introduction

Histamine has been identified in the brain of many species, including man (see Green *et al.*, 1978 for review). The amine does not readily penetrate the brain from the cerebral circulation (Schayer & Reilly, 1970), and therefore its occurrence, and the identification of specific enzymes for its synthesis (histidine decarboxylase) and catabolism (histamine N-methyl transferase) in brain tissue of various mammalian species including man (Barbin *et al.*, 1980), supports the contention that locally synthesized histamine mediates specific functions in the tissue. Within the brain histamine appears to be localized in several pools. A rapidly turning over pool is believed to comprise neuronal histamine; a second more slowly turning over pool may comprise mast cell histamine (Garbarg *et al.*, 1980; Maayani *et al.*, 1982; Hough *et al.*, 1984a,b).

Further support for the view that histamine may mediate specific functions in brain derives from clear evidence for histamine receptors in the tissue; both H_1- and H_2-receptors have been identified. Evidence for H_1-receptors is based on [^{3}H]-mepyramine binding studies (Hill *et al.*, 1978: Tran *et al.*, 1978), histamine-stimulated glycogenolysis (Quach *et al.*, 1980), histamine-stimulated phosphatidylinositol turnover (Daum *et al.*, 1983), and adenosine 3':5'-cyclic monophosphate (cyclic AMP) accumulation studies (Palacios *et al.*, 1978). Evidence for H_2-receptors in brain derives from studies on histamine-stimulated adenylate cyclase (Hegstrand *et al.*, 1976; Green *et al.*, 1977; Kanof & Greengard, 1979), histamine-stimulated cyclic AMP accumulation (Palacios *et al.*, 1978), [^{3}H]-tiotidine binding (Gajtkowski *et al.*, 1983) and from electrophysiological studies in rat hippocampal slices (Haas & Konnerth, 1983). In addition, Schwartz and co-workers have presented evidence for an H_3-autoreceptor mediating feedback inhibition of neurotransmitter histamine release (Arrang *et al.*, 1983).

If histamine and its receptors can be found in brain, what functions might histamine control in the tissue? Studies to this end would be facilitated by the

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availability of suitable selective receptor agonists and antagonists. While the advent of selective H_2-receptor antagonists has helped to clarify the role of histamine in gastric acid secretion (Black *et al.*, 1972), the currently available agents do not readily penetrate the brain, limiting their utility in investigations on the functions of histamine acting at central H_2-receptors. In this paper, the identification of 2-[3-[3-(piperidinomethyl) phenoxy]propylamino]benzthiazole, zolantidine (SK&F 95282, structure shown in Figure 1) as a potent selective brain-penetrating histamine H_2-receptor antagonist is described.

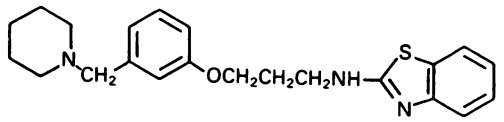

Figure 1 Structure of zolantidine (SK&F 95282)

Methods

Antagonism at atrial H_2-receptors

Guinea-pigs of either sex, weighing 400–700 g, were killed by cervical dislocation and a triangular piece of right atrium (including the sino-atrial node) was removed as quickly as possible. This atrial strip, mounted in an acrylic holder, was suspended in a 15 ml bath containing McEwan's solution (concentrations, mM: sodium chloride 130, potassium chloride 5.6, sodium acid phosphate dihydrate 2.5, glucose 11.1, sucrose 13.1, sodium bicarbonate 25, calcium chloride 2.2) at 34°C and gassed with 95% O_2:5% CO_2. The contraction frequency was recorded continuously on a potentiometric chart recorder; the signal was the smoothed output of an instantaneous (reciprocal of interval) rate meter which had been triggered by a force transducer attached to the muscle. The muscle was loaded to 300 mg tension. A cumulative dose-response curve for histamine was constructed using dose increments based on a geometric progression with a common ratio of 4. A second dose-response curve to histamine was constructed after addition of the antagonist (30 min pre-incubation). The degree of antagonism was measured as a dose-ratio (DR), defined as the displacement of the treated dose-response curve from the control. From the dose-ratios determined with different concentrations of the antagonist, pA_2 values were calculated (Arunlakshana & Schild, 1959).

Antagonism at rat uterus H_2-receptors

Female rats weighing 200–250 g in early oestrus were killed and the uterine horns dissected out and the surrounding fat and mesenteric attachments were removed. The uterus was suspended in a 10 ml isolated organ bath at 34°C containing McEwan's solution and gassed with 95% O_2: 5% CO_2. The uterus was attached to a Harvard isometric transducer connected to a Harvard transducer amplifier and recorded on a potentiometric chart recorder. The uterus was loaded to 0.5 g tension. The uterus was depolarized by KCl (37 mM) and a cumulative dose-response relaxation curve for histamine was constructed using dose increments based on a geometric progression with a common ratio of 3. A second dose-response curve to histamine was constructed after addition of the antagonist (30 min pre-incubation). The degree of antagonism was measured as a dose-ratio (DR), defined as the displacement of the treated dose-response curve from the control. From these dose-ratios determined with different concentrations of the antagonist, pA_2 values were calculated (Arunlakshana & Schild, 1959).

Inhibition of [^{3}H]-tiotidine binding in guinea-pig cortex tissue

The method used was essentially that of Gajtkowski *et al.* (1983). Tissue was prepared by centrifuging (50000 *g* for 10 min) a 30-volume homogenate of guinea-pig cerebral cortex in 50 mM Na-K phosphate buffer (pH 7.4) and resuspending the pellet in the original volume of buffer: all operations at 4°C.

Cortex tissue (100 µl of resuspension) and [^{3}H]-tiotidine (final concentration 2 nM) were incubated together with the test substance (total volume 500 µl) at 20°C for 30 min. All dilutions were in 50 mM Na-K phosphate buffer (pH 7.4). The incubations were terminated by addition of 5 ml ice-cold buffer, with immediate filtration through Whatman GF/B glass fibre filters (pre-soaked in buffer containing 1% bovine serum albumin) under vacuum of 18 psi on a Millipore 12-place filtration block with immediate washing of the filters with a further 3 × 5 ml buffer. Filters were removed and placed in scintillation vials with 15 ml Picofluor 15 scintillant, shaken vigorously to break up the filters, and stored for at least 2 h at room temperature before counting.

Inhibition data were processed by least squares fit to a logistic equation (ALLFIT program: De Lean *et al.*, 1978); the IC_{50} concentration for the antagonist was converted into a K_i value using the equation $K_i = IC_{50}/(1 + [^3H]\text{-tiotidine concentration}/[^3H]\text{-tiotidine } K_D)$. A K_D value of 14.6 nM was used in the calculations, determined in a saturation experiment (data not shown) cf. 17 nM (Gajtkowski *et al.*, 1983).

Inhibition of histamine-stimulated adenylate cyclase activity

The procedure of Green & Maayani (1977) was used to prepare the tissue. Dorsal hippocampus from male Dunkin-Hartley guinea-pigs (approx. 300 g) was homogenized in 100 volumes of ice-cold 5 mM Tris-HCl (pH 7.4), containing 0.32 M sucrose, 1 mM EGTA, and 1 mM dithiothreitol. Aliquots of the homogenate were used in the incubations within 30 min. Guinea-pig cardiac ventricle homogenates were prepared from heart in a similar manner.

Adenylate cyclase activity was measured by a modification of the method of Johnson & Mizoguchi (1977). The assay system contained 75 mM Tris-HCl (pH 7.4), 10 mM GTP, 2 mM $MgCl_2$, 1 mM IBMX, 5 mM phosphocreatine, 20 units creatine phosphokinase, 1 mM cyclic AMP, 0.30 mM EGTA, 0.1 mM sucrose, 0.3 mM dithiothreitol, 1 mM ATP, agonists and antagonists as appropriate, and enzyme preparation (50 μg protein in 50 μl), total volume 250 μl. After a pre-incubation period of 5 min at 30°C in a shaking water-bath, the reaction was started by addition of [α-^{32}P]-ATP (10–40 Ci mmol^{-1}, 0.5 μCi in 20 μl) and continued for a further 10 min. The reaction was terminated by addition of 100 μl 2% sodium dodecylsulphate and the tubes were transferred to ice. [^{32}P]-cyclic AMP was isolated by the dual column method (dowex AG 50W-X4 and alumina; Salomon *et al.*, 1974), after each tube had been spiked with 0.35 mg each of ATP and cyclic AMP, and approx. 7000 d.p.m. [^{3}H]-cyclic AMP to monitor recovery, then diluted to 1 ml with water. The eluate containing the product cyclic AMP was collected in a scintillation vial, and 15 ml pico-fluor 15 added for counting of ^{3}H and ^{32}P. Enzyme activity was calculated from the amount of [^{32}P]-cyclic AMP formed, corrected for recovery of [^{3}H]-cyclic AMP (routinely 60–70%), and is expressed as pmol cyclic AMP min^{-1} mg^{-1} protein. Incubations were normally carried out in triplicate. Data analysis (EC_{50} values for agonist concentration-response curves, IC_{50} values for antagonists) was performed using the ALLFIT routine for least squares fit to a logistic function. The IC_{50} value was converted to an inhibition constant K_i using the equation $K_i = IC_{50}/(I + A/EC_{50})$ [Kanof & Greengard (1978)], where A is the fixed agonist concentration.

Inhibition of H_2-receptor-mediated cyclic AMP accumulation in guinea-pig hippocampal slices

The method used is essentially that of Palacios *et al.* (1978). The specific H_2-receptor agonist dimaprit was used to stimulate H_2-receptor-mediated cyclic AMP accumulation. Four guinea-pigs were killed by cervical dislocation and their hippocampi removed and washed in ice-cold buffer (Krebs Ringer bicarbonate buffer, pH 7.4, equilibrated with 95% O_2:5% CO_2). They were blotted dry, weighed and sliced in two directions by a McIlwain tissue chopper set at 250 μm. Slices were suspended in Krebs buffer and incubated, with one change of buffer, at 37°C under a continuous flow of O_2:CO_2 for 15 min. This allowed the slices to stabilize and reduced basal cyclic AMP levels from the high post-mortem levels.

Slices were collected and resuspended 1:10 (w/v) in warm Krebs buffer: 200 μl of the stirred suspension was dispensed into incubation flasks containing Krebs buffer. After being gassed and stoppered, flasks were preincubated at 37°C in a shaking water bath for 10 min. Antagonists, when present, were included in the pre-incubations. Incubations were started by the addition of agonist dimaprit at the required concentration. Flasks were regassed, stoppered and incubated for 15 min. The final volume was 4 ml, and all incubations were in quadruplicate. The incubation was terminated by homogenization of samples (Polytron setting 5, 5 s) and placing them in a boiling water bath for 10 min. After cooling, 100 μl of each sample was assayed in duplicate for cyclic AMP using a commercial assay kit. The antagonist was checked at the highest concentration used for its effect on the cyclic AMP protein binding assay (effect on 1 pmol cyclic AMP standard was measured).

D.p.m. data were converted into pmol of cyclic AMP using the standard curve, and cyclic AMP data was analysed by 'ALLFIT' (De Lean *et al.*, 1978) yielding estimates of the EC_{50} for agonist concentration response curves or IC_{50} for antagonist inhibition curves. From IC_{50} experiments the antagonist K_i was calculated from the relationship:-

$$K_i = IC_{50}/(1 + \text{dimaprit conc.}/\text{dimaprit } EC_{50})$$

Brain/blood ratio in the male rat

The method developed by Griffiths and co-workers was used (Brown *et al.*, 1986). Briefly, a saline solution of [^{14}C]-zolantidine, labelled at the benzylic methylene was administered by i.v. bolus (2 ml) followed by an i.v. infusion (approx. 2.0 ml h^{-1}) of the dose solution via the femoral vein of the rat. Where anaesthetized animals were used, this was achieved by intraperitoneal injection of urethane solution (25% w/o, 1.5 g kg^{-1}).

The infusion was maintained until the ^{14}C-radioactivity in the blood was at a plateau (approx. 2 h). This was done by monitoring the level of total radioactivity in the blood during the course of the infusion by taking blood samples from the tail vein at predetermined times and assaying them for radioactivity by liquid scintillation counting. At the end of the infusion, the rat was exsanguinated by cardiac puncture. The brain was removed, rinsed in chilled saline and then placed

on a refrigerated glass plate. It was cut down the midline, and one half was dissected into 11 regions of interest. The tissues were weighed and solubilized in Soluene-100 (1 ml for approximately 150 mg tissue, 37°C, overnight). Glacial acetic acid (50 μl for 1 ml Soluene) was added, before the solutions were assayed for radioactivity, in order to prevent chemiluminescence. Blood samples were counted directly in Picofluor 15 scintillant (10 ml) and solubilized brain samples were counted in Dimilume-30 scintillant (15 ml).

The blood concentration at the end of the infusion and the concentration in the brain were calculated. The brain:blood concentration ratio was estimated, this being a measure of the extent to which the compound (and its metabolites) were accumulating in the brain with respect to the blood. [^{3}H]-inulin was originally included as a blood marker, in order to allow a correction to be made for residual blood present in the brain at the time of excision. However, it has become apparent that this blood marker overestimates the amount of blood in the brain, and a value of 15 μl blood g^{-1} brain tissue (derived from experiments using [^{14}C]-methylhydroxyinulin) has been used to correct the brain concentrations. All concentrations quoted are expressed as molar equivalents of parent compound.

Materials

Zolantidine dimaleate, dimaprit dihydrochloride SK&F 9344 temelastine; 2-[4-(3-methyl-5-bromopyrid-2-yl) butylamino]-5-[(6-methylpyrid-3-yl) methyl]-4-pyrimidone) and cimetidine were synthesized in the Medicinal Chemistry Department at SK&F. Histamine acid phosphate was from BDH. [^{14}C]-zolantidine was synthesized in the Department of Synthetic and Isotope Chemistry at SK&F.

[^{3}H]-tiotidine was supplied by New England Nuclear; all other radiochemicals and the cyclic AMP assay kits were supplied by Amersham International plc. Pico-fluor 15 and Soluene-100 were supplied by United Technologies Packard.

Results

Guinea-pig right atrium H_2-receptor antagonism

Zolantidine produced a concentration-related dextral shift of the histamine concentration-response curve in the guinea-pig right atrium. However, the slope of the Schild plot derived from these studies was 0.71 (95% confidence limits 0.62–0.79), and since this was significantly different from unity only an apparent pA_2 7.46 (95% confidence limits 7.33–7.62) could be calculated. The compound did not depress the maximum response to histamine but elicited some negative chronotropic activity (ca. 16 beats min^{-1} at a concentration 28 fold that giving dose-ratio = 2).

Rat uterus H_2-receptor antagonism

SK&F 95282 also produced a concentration-related dextral shift of the histamine concentration-response curve in the rat uterus. The slope of the Schild plot (0.75) was significantly different from unity (95% confidence limits 0.53–0.96) and an apparent pA_2 7.26 (95% confidence limits 7.04–7.64) was calculated.

Inhibition of [^{3}H]-tiotidine binding in brain

Zolantidine was tested over a range of concentrations against the binding of 2 nM [^{3}H]-tiotidine to guinea-pig cerebral cortex tissue. At this concentration of [^{3}H]-tiotidine, specific cerebral binding determined with unlabelled cimetidine (100 μM) comprised over 40% of total binding. Zolantidine displaced [^{3}H]-tiotidine binding with IC_{50} 76 ± 7 nM (data pooled from 3 experiments), corresponding to K_i 67 ± 6 nM (pK_i 7.17). A representative displacement curve is shown in Figure 2. Displacement to non-specific binding levels was achieved at ca. 5 μM. Zolantidine did not displace filter binding of [^{3}H]-tiotidine, which in any case accounted for only ca. 10% of total binding. In a similar experiment, zolantidine also displaced [^{3}H]-tiotidine binding to guinea-pig striatum tissue, K_i 68 ± 22 nM (data not shown).

Inhibition of histamine-stimulated adenylate cyclase

Zolantidine inhibited the stimulation of adenylate

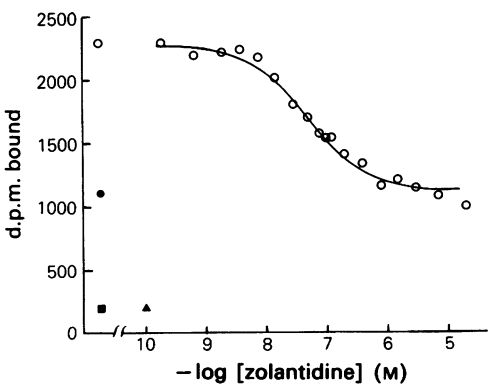

Figure 2 Competition by zolantidine for [^{3}H]-tiotidine binding to guinea-pig cortex membranes. Points are means of triplicates; individual data points were within 3% of the mean. Logistic fit to the data gave IC_{50} 56 ± 4 nM, slope − 0.89 ± 0.05. Zolantidine competed with [^{3}H]-tiotidine binding to the non-specific binding level set by 100 μM cimetidine (●); FB (fiter binding) (■); FB + 10 μM zolantidine (▲).

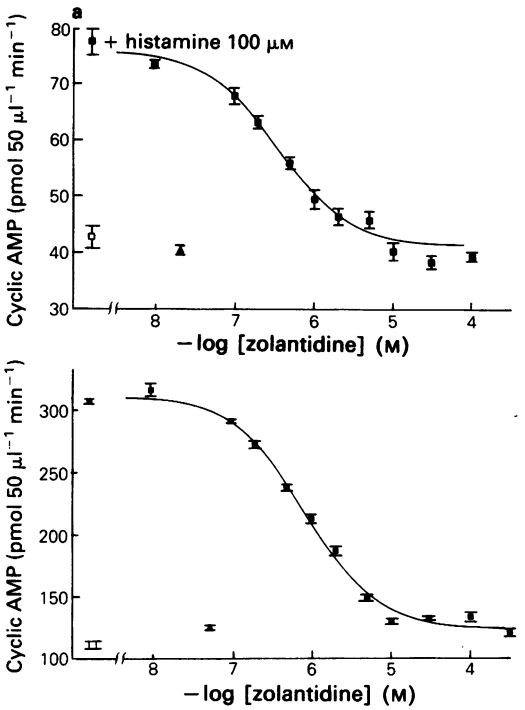

Figure 3 Inhibition by zolantidine of adenylate cyclase stimulated by histamine (100 μM). (a) Guinea-pig hippocampal homogenate; (b) guinea-pig cardiac ventricle homogenate. Incubations were in triplicate; means are shown with s.e.mean indicated by vertical lines. Basal adenylate cyclase activity (□) was not significantly affected by zolantidine (100 μM, ▲)

cyclase by 100 μM histamine in guinea-pig hippocampal homogenate in a concentration-dependent manner (Figure 3a). An IC_{50} value of 340 ± 60 nM was obtained, corresponding to a K_i value of 42 ± 7 nM (pK_i 7.4). Zolantidine also inhibited histamine-stimulated adenylate cyclase in a guinea-pig cardiac ventricle homogenate (Figure 3b). An IC_{50} value of 840 ± 60 nM was obtained, corresponding to a K_i value of 52 ± 4 nM, (pK_i 7.3). Histamine EC_{50} values of 14 μM (hippocampus) and 6.6 μM (ventricle) were used to convert IC_{50} into K_i values.

Dimaprit-stimulated cyclic AMP accumulation

The effect of zolantidine on dimaprit-stimulated cyclic AMP accumulation in guinea-pig hippocampal slices is shown in Figure 4. A Schild plot constructed from concentration-ratios obtained for dimaprit in the presence of 0.048, 0.1, 0.3 and 3 μM zolantidine had

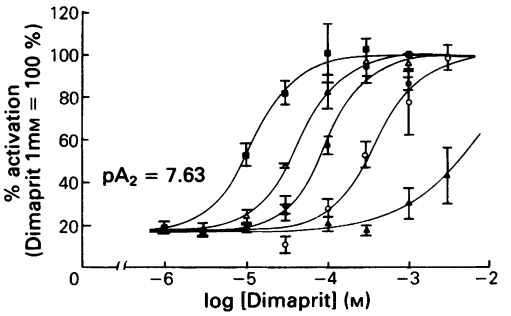

Figure 4 Stimulation by dimaprit of cyclic AMP accumulation in guinea-pig hippocampal slices in the absence (■) and presence of zolantidine: 0.048 μM (△); 0.1 μM (●); 0.3 μM (○); 3.0 μM (▲). Incubations were in quadruplicate; mean values are shown with s.e.mean indicated by vertical lines.

a slope of 1.34 (95% confidence limits 1.25–1.43), significantly greater than unity, and gave an apparent pA_2 of 7.63 (95% confidence limits 7.58–7.68). The compound did not depress the maximum response to dimaprit. When zolantidine was tested against a fixed (100 μM) concentration of dimaprit, an IC_{50} of 118 ± 29 nM (mean ± s.e. for data pooled from 3 experiments), corresponding to K_i 18.5 ± 4.5 nM (pK_i 7.73), was obtained (data not shown). An EC_{50} of 18.6 μM for dimaprit was used in calculation of K_i values.

Brain-blood ratio for [^{14}C]-zolantidine in the rat

[^{14}C]-zolantidine was evenly distributed in brains of anaesthetized rats, with the exception of the brain stem where the concentration was 10–20% higher than that found in other areas. The brain/blood ratio for whole brain was 1.45 ± 0.60 (n = 4). The distribution of radioactivity in brains from conscious rats was even, with a brain/blood ratio of 1.34 ± 0.32 (n = 4). In a further study in conscious rats, it was ascertained that under the conditions used, >90% of circulating radioactivity represented zolantidine (shown by t.l.c.), and that radioactivity extracted from brain yielded only [^{14}C]-zolantidine on analysis by t.l.c..

In similar experiments, the brain/blood ratios for [^{3}H]-mepyramine (which penetrates readily) and [^{14}C]-SK&F 93944, (an H_1-receptor antagonist which penetrates poorly), were 3.16 ± 1.48 (n = 8) and 0.02 ± 0.004 (n = 12) respectively (Brown *et al.*, 1986).

Receptor selectivity

Antagonist activity of zolantidine at other peripheral and central receptors is shown in Table 1. The

compound was also tested as an inhibitor of the histamine synthesizing enzyme (histidine decarboxylase), and the histamine catabolizing enzyme (histamine N-methyltransferase). Data are shown in Table 1, and indicate that zolantidine was at least 30 fold more potent at H_2-receptors relative to other sites.

Discussion

Selective H_2-receptor antagonists have been available for over a decade and although such compounds have helped greatly to clarify the role of peripheral histamine, for example in gastric acid secretion, their low ability to penetrate the blood-brain barrier has limited their utility in investigating functions mediated by H_2-receptors in brain. Such studies would be greatly facilitated by a brain-penetrating agent, and zolantidine is characterized here as a potent selective brain-penetrating H_2-receptor antagonist suitable for *in vivo* experimentation.

The known potent selective H_2-receptor antagonists, for example cimetidine, ranitidine, tiotidine and SK&F 93479 (Ganellin, 1982), appear to have poor ability to penetrate into the brain, e.g. cimetidine has a brain/blood ratio measured at steady state of 0.036 ± 0.006, $n = 8$ (R. Griffiths, unpublished observations). In order to design an H_2-receptor antagonist which combines potency and selectivity with improved entry into brain, studies were carried out to identify physicochemical properties of existing antagonists which should be modified to increase brain penetration in analogues. Biological membranes are generally considered to be primarily lipoidal and permeable to uncharged chemical species, and the relative ability of these species to penetrate membranes is thought to depend on their lipid solubilities and size. In searching for a suitable measure of lipid

Table 1 Receptor selectivity studies on zolantidine

Assay system	*Receptor*	*Inhibition constant*	*Ref. to Method*
Histamine stimulated contraction of guinea-pig ileum	H_1-histamine	pA_2 5.21	
[^{3}H]-mepyramine binding to guinea-pig cerebral cortex	H_1-histamine	pK_i 5.1	Tran *et al.* (1978)
Carbachol-stimulated contraction of guinea-pig ileum	Muscarinic	pA_2 5.3	
[^{3}H]-QNB binding to guinea-pig cerebral cortex	Muscarinic	pK_i 5.9	Snyder *et al.* (1975)
Isoprenaline-stimulated chronotropism in the guinea-pig right atrium	β-Adrenoceptor	$pA_2 < 5.2$	
Dopamine-stimulated adenylate cyclase in rat striatal homogenate	D_1-dopamine	$pK_i < 5.0$	Kebabian & Calne (1979)
[^{3}H]-spiperone binding to rat striatal tissue	D_2-dopamine	pK_i 5.9	Quik *et al.* (1978)
[^{3}H]-prazosin binding to rat cerebral cortex	α_1-Adrenoceptor	pK_i 4.9	Greengrass & Bremner (1979)
[^{3}H]-yohimbine binding to rat cerebral cortex	α_2-Adrenoceptor	pK_i 5.6	Cheung *et al.* (1982)
[^{3}H]-5-HT binding to rat frontal cortex	5-HT_1	pK_i 5.8	Martin & Sandersbush (1982)
[^{3}H]-spiperone binding to rat frontal cortex	5-HT_2	pK_i 5.3	Peroutka & Snyder (1980)
[^{3}H]-GABA binding to rat brain tissue preparation	$GABA_A$	pK_i 3.4	Bowery *et al.* (1983)
[^{3}H]-baclofen binding to rat brain tissue preparation	$GABA_B$	pK_i 2.9	Bowery *et al.* (1983)
[^{3}H]-diazepam binding to rat cerebral cortex tissue	Benzodiazepin	pK_i 4.9	Braestrup & Squires (1977)
Guinea-pig brain HMT catalysed methylation of histamine with [^{14}C-Me] SAM	Histamine N-methyl transferase	pK_i 6.6	Dent *et al.* (1982)
Guinea-pig brain HDC catalysed decarboxylation of [^{3}H]-histidine	Histidine decarboxylase	$pK_i < 3.5$	Keeling (1981)

solubility, the partition coefficients of selected H_2 antagonists were determined in different solvent systems and compared with those of clonidine, imipramine and mepyramine, which readily cross the blood-brain barrier. Partition coefficients (P) measured in the octanol/water system, corrected for ionisation, showed no correlation with the equilibrium brain/blood ratios, while the ratio between the octanol/water and cyclohexane/water P values showed a good correlation (Young *et al.*, personal communication). This suggested that brain penetration might be increased by reducing the ratio of partition coefficients and, since this parameter is related to the overall hydrogen-bonding ability of a compound (Seiler, 1974), attempts were made to minimize the hydrogen-bonding ability of known H_2-receptor antagonists by structural manipulation. This approach led to the synthesis of a number of novel, brain-penetrating H_2-receptor antagonists, the most notable of which is zolantidine. Radiolabelled zolantidine was shown to penetrate the blood-brain barrier after peripheral administration in the anaesthetized rat with a steady state brain/blood ratio of 1.45, which is about half that of mepyramine determined under similar conditions, and 40 times that of cimetidine.

The method of drug administration used in these studies was designed to achieve steady state blood concentrations of zolantidine as soon as possible. At steady state, drug in the blood would be expected to be in equilibrium with the major tissues in the body, which in the case of a relatively lipophilic compound such as zolantidine, should include the brain. Even if true equilibrium is not achieved between blood and brain it is highly likely that the drug concentration time course in the brain would, if anything, lag behind that in the blood, and give rise to an underestimate of the brain:blood ratio in these experiments.

The proportion of zolantidine bound in rat serum, as measured by equilibrium dialysis *in vitro*, is approximately 96.4%–94.5% over the concentration range relevant to these experiments (0.25–12.5 μM) (E.A. Brown, unpublished observations). In the absence of any other considerations, at true equilibrium the concentration of free drug ought to be the same on either side of the blood-brain barrier. As the concentration of free zolantidine in the serum is low (5% of the total concentration), the high value of the brain: blood ratio in these experiments is probably an indication of a high affinity of the compound for brain tissue.

The potency of zolantidine has been demonstrated at both peripheral and central H_2-receptors. Good agreement was found between data obtained from peripheral (guinea-pig right atrium and rat uterus) and central H_2-receptors (guinea-pig hippocampal adenylate cyclase stimulation and cyclic AMP accumulation, and [^{3}H]-tiotidine binding to guinea-pig cortex and striatal tissue). There was no evidence for tissue-related pharmacological differences in the H_2-receptor using zolantidine as antagonist. The high potency of zolantidine at H_2-receptors is indicated by comparison with cimetidine which has pA_2 6.1 on the atrium (Brimblecombe *et al.*, 1975), and pK_i 6.3 when tested against [^{3}H]-tiotidine binding to central H_2-receptors (Gajtkowski *et al.*, 1983), pA_2 6.22 as an antagonist of histamine-stimulated adenylate cyclase in guinea-pig hippocampal homogenates (Green *et al.*, 1977), and pK_i of 6.2 as an antagonist of impromidine-stimulated cyclic AMP accumulation in guinea-pig hippocampal slices (Dam Trung Tuong *et al.*, 1980).

The ability of zolantidine to antagonize potently the accumulation of cyclic AMP in brain slices was of particular interest. Several groups of researchers, (Green & Maayani, 1977; Kanof & Greengard, 1978; 1979) have shown that some anti-depressant, antipsychotic and other drugs can antagonize potently the stimulation of adenylate cyclase by histamine in guinea-pig brain homogenates, while Schwartz and co-workers (Dam Trung Tuong *et al.*, 1980) have demonstrated that these drugs are up to 100 fold less potent as antagonists in the slice system; Schwartz and co-workers suggest that the difference may be due to modified discriminatory characteristics of the receptors upon cell disruption. However, it has been suggested by Angus & Black (1980) that such discrepancies between data obtained from broken or intact cell preparations may be due to a secondary intracellular action of the agent such as inhibition of phosphodiesterase activity in the intact system. If so, this would be more likely for brain penetrating agents such as antidepressants whose physicochemical properties allow them to diffuse across cell membranes. It is therefore a key observation that, in contrast to the action of antidepressants, zolantidine was found to be a potent competitive antagonist against H_2-receptor-mediated cyclic AMP accumulation in guinea-pig hippocampal slices, indicating its utility as an H_2-receptor antagonist in intact tissues. This is also indicated by the ability of zolantidine to antagonize potently the actions of histamine at H_2-receptors in the guinea-pig right atrium and rat uterus, although at present no explanation can be provided for low slopes of the Schild plots obtained on these tissues.

To be a useful pharmacological tool, an agent should be selective as well as potent. Zolantidine has been shown to be a selective H_2-receptor antagonist relative to a range of other peripheral and central receptors. As shown in Table 1 it was at least 30 fold more potent at H_2-receptors than at other sites investigated. Additionally, at concentrations up to 300 μM, zolantidine does not inhibit the histamine synthesizing enzyme histidine decarboxylase (from guinea-pig brain). It was found to be a competitive inhibitor of the

histamine catabolising enzyme in brain, histamine N-methyltransferase (HMT), with K_i of 0.27 μM (pK_i 6.6), compared with H_2-receptor pA_2 of 7.8 (cyclic AMP). Inhibition of HMT by zolantidine might tend to raise brain histamine levels but the higher affinity of the compound at H_2-receptors should adequately negate this effect.

Thus, zolantidine represents a potent selective H_2-receptor antagonist whose brain:blood ratio indicates that it is able to penetrate the brain after peripheral administration and should help to define functions mediated by H_2-receptors in the brain. What information on possible functions is already available? Certainly, a neurotransmitter function can be envisaged, based on histamine turnover studies (Pollard *et al.*, 1974; Dismukes & Snyder, 1974; Hough *et al.*, 1984a; Nishibori *et al.*, 1984). For example, Nishibori *et al.* (1984) observed half-lives of 8–87 min for the rapidly turning over pool of histamine in various regions of rat, guinea-pig and mouse brain. The observation of measurable quantities of histidine decarboxylase in brain also suggests an ongoing requirement for histamine (Schwartz *et al.*, 1980); in contrast, some other tissues, for example heart, store relatively large quantities of the amine (Beaven & Horakova, 1978), but have extremely low quantities of histidine decarboxylase (Watanabe & Wada, 1983).

In addition to the above, there appear to be histaminergic pathways in the brain. Ascending pathways with cell bodies localised in the mesencephalic reticular formation and posterior mamillary bodies appear to innervate ipsilaterally much of the di- and telencephalon (Schwartz *et al.*, 1982). A descending projection to the mid-brain and lower brain stem has also been suggested (Watanabe *et al.*, 1984). Thus the histaminergic neuronal system resembles those of noradrenaline and 5-hydroxytryptamine in consisting of a relatively small number of cells projecting widely to large areas of forebrain and hindbrain and may indicate a role for histamine in control of the overall levels of functioning of the forebrain, for example in control of sleep/wakefulness, rather than the transmission of discrete information. Further recent evidence comes from two groups of researchers using the hippocampal slice preparation *in vitro*. Haas & Konnerth (1983) have shown that, histamine enhances the response of pyramidal cells to excitatory inputs, such as current injection or the application of excitatory amino-acids, and Olianas *et al.* (1984) have demonstrated that histamine increases the firing rate of CA3 pyramidal cells. In both cases, the effects were blocked by histamine H_2-receptor antagonists. Parallel experiments with tissue homogenates showed a correlation between the electrophysiological events and the activation of adenylate cyclase (Olianas *et al.*, 1984). Furthermore, other studies with adenylate cyclase and [^{3}H]-tiotidine binding have indicated clearly the presence of histamine H_2-receptors in areas innervated by the ascending histaminergic system (Hegstrand *et al.*, 1976; Green *et al.*, 1977; Norris *et al.*, 1984).

Additionally, H_2-receptors have been shown to be associated with the action of histamine on the cerebral vasculature, e.g. accumulation of cyclic AMP in microvessels (Karnushina *et al.*, 1980) and vasodilatation of pial arteries (Wahl & Kuschinsky, 1979; Edvinsson *et al.*, 1983; Calcutt *et al.*, unpublished observations). Interestingly, mast cells may provide a source of histamine to act upon cerebrovascular receptors; a slowly turning over pool of histamine in brain may comprise mast cell histamine (Garbarg *et al.*, 1980; Maeyama *et al.*, 1983; Hough *et al.*, 1984a,b), and microvessel preparations described by Head *et al.* (1980), were observed under the microscope to contain electron-dense cytoplasmic granules having the appearance of the type II mast cells of Ibrahim (1974). In addition, mast cells have been identified directly in the mammalian central nervous system of several species including man using staining procedures (Ibrahim, 1974; Edvinsson *et al.*, 1977; Goldschmidt *et al.*, 1984) often with a perivascular localization, and suggesting that mast cell histamine may have some role in control of cerebral blood flow. Degranulation of such mast cells would release histamine on the brain side of the blood-brain barrier, and reversal of the actions of this histamine would presumably require a brain-penetrating antagonist. If so, one can envisage that such an antagonist may be of value in treating some forms of vascular headache (Gross, 1982).

Thus there are potential functions of histamine in brain mediated by H_2-receptors. The availability of a brain penetrating H_2-receptor antagonist should help to clarify these functions further.

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