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Genetic heterogeneity of histamine H_2-receptors in the mouse vas deferens

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1 The ability of histamine to inhibit the overall contractile ('twitch') response of the isolated vas deferens of the mouse to electrical field stimulation (64 V pulse, 1 ms pulse width, frequency 0.2 Hz) was studied in nine inbred mouse strains. The strains were also characterized in terms of the potency of the histamine H_2-receptor antagonist cimetidine in its inhibition of histamine-mediated effects. An apparently bimodal inter-strain variation (8–10 fold) in both characteristics was encountered, with three strains (SWR, A2G and C57BL/10ScSn) relatively sensitive (S) to both agonist and antagonist actions, and six (C3H, A, C57/BL6, DBA/2, Balb/C and 129/Sv) relatively insensitive (IS).

2 These strain differences were independent of extracellular calcium concentration in the range 1.25–5 mM, and also independent of the frequency of tissue stimulation over the range 0.2–6.4 Hz.

3 Representative S (SWR and A2G) and IS (DBA/2 and C3H) mouse vasa were also characterized in terms of their sensitivity to the agonist actions of dimaprit and the antagonist actions of tiotidine. In the S strain tissues, dimaprit produced 50% inhibition of the twitch response at 4.6–1.8 μM (mean ± s.d.) and was able to elicit complete inhibition of the twitch response at concentrations greater than 100 μM, whereas 48.7 ± 11.9 μM dimaprit was required to produce 50% inhibition of the twitch response in tissues from IS mice. In addition, the agonist actions of dimaprit were incomplete in the latter tissues, the drug eliciting no more than 75% inhibition of the twitch response at concentrations in the range 300–1000 μM. Tiotidine produced competitive antagonism of the actions of both histamine and dimaprit, the strain differences being of the same magnitude as those observed for cimetidine.

4 Mating of a representative S (SWR) and IS (129/Sv) strain produced F_1 mice with intermediate histamine and cimetidine sensitivities relative to the parental strains. A backcross of male F_1 to female IS mice produced progeny displaying a range of histamine and cimetidine sensitivities representative of those seen in tissues from F_1 and IS parental animals, however, the data were not bimodal. Thus, the backcross data provided no evidence to support single gene inheritance of histamine sensitivity and might suggest that more than one gene is responsible for these differences between S and IS mice.

Introduction

It is a well documented fact that a large number of drugs display a wide variation, both between and within species in their beneficial pharmacological effects, and also in their propensity to cause adverse reactions. There is considerable evidence that some of this variability can be attributed to genetic factors operating at the level of pharmacokinetic (i.e. 'pre-receptor') events (Idle & Smith, 1979; Al-Dabbagh *et al.*, 1981), however, relatively little attention has been paid to the factors controlling pharmacodynamic events. Such studies as are available in the literature would indicate that genetic variability in the characteristics of receptor populations may be considerable. The investigations of Henderson & Hughes (1976), Waterfield *et al.* (1978) and Szerb & Vohra (1979) have produced convincing evidence that the sensitivity of mouse vas deferens to both endogenous and exogenous opiate agonists displays marked inter-strain variability. Similarly, Hall *et al.* (1978) have demonstrated that the nicotinic acetyl-

choline receptor in the central nervous system of *Drosophila melanogaster* displays genetic variation in its isoelectric focusing point and that this variation at the molecular level is manifested as variable sensitivity to the lethal effects of nicotine in the intact insect.

We have recently reported a preliminary survey of the degree of interstrain variation in the characteristics of the histamine H_2-receptor of the mouse vas deferens (Lush *et al.*, 1982). Nine inbred strains were characterized both in terms of the potency of histamine in its inhibition of the contractile response of the isolated vas deferens to electrical field stimulation and in terms of the ability of cimetidine, a selective histamine H_2-receptor blocking agent, to antagonize histamine-mediated effects. This experimental system was selected as a model for the study of the influence of genetic factors on receptor function on a number of criteria. The mouse vas deferens appears to be unique in displaying only H_2-receptor-mediated effects to histamine (Marshall, 1978; Vohra, 1979), vasa deferentia from other species (rat, rabbit and guinea-pig) all display varying proportions of mutually antagonistic H_1- and H_2-receptor mediated effects (Vohra, 1981). The mouse tissue thus provides an opportunity to examine the histamine H_2-receptor under conditions relatively free of other variables. However, the response to histamine is markedly influenced by extracellular calcium concentration (Vohra, 1979), and thus a study of the response to histamine in isolation could give rise to spurious 'receptor' differences actually due to, for example, differences in the utilization of calcium by smooth muscle. A full characterization of the histamine H_2-receptor-mediated response thus requires the determination of parameters related to receptor affinity for both an agonist and an antagonist. Since an apparent affinity constant for an antagonist is derived from dose-ratios rather than the actual tissue responses, it will be less sensitive to variation at levels other than the receptor site.

The results of preliminary studies of the characteristics of the mouse vas deferens histamine H_2-receptor revealed eight to ten fold inter-strain variation with respect to both agonist and antagonist parameters. This variation was apparently bimodally distributed, suggesting that the histamine H_2-receptor present in this preparation is controlled by a single gene of major effect. It is the purpose of this report to present a fuller description of the pharmacological and genetic characteristics of this strain variability; the strains designated as sensitive (S) and insensitive (IS) were studied in terms of the agonist action of histamine and dimaprit, and in terms of the antagonist potencies of cimetidine and tiotidine, the latter compound being the highest affinity histamine H_2-receptor antagonist currently available (Yellin *et al.*, 1979). The results of mating a representative S and IS strain, followed by a backcross of F_1 mice to a parental IS strain in order to test for monogenetic inheritance of S/IS characters is also described.

Methods

In vitro *experiments*

Except where stipulated, all experiments were carried out in a bathing solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11; there being no magnesium included in order to improve the response to electrical stimulation (Hughes *et al.*, 1975). The bathing solution was gassed with 95% O_2 and 5%CO_2 and maintained at 37°C in all experiments. Electrical stimulation of tissues was achieved through two vertical platinum gutter electrodes delivering single 1 ms square wave pulses at 64 V at a frequency of 0.2 Hz. Contractions were recorded isometrically via a Grass FTO3C force displacement transducer connected to a Grass 79D Polygraph recorder. The Polygraph d.c. driver amplifier was interfaced with an analogue-to-digital converter which displayed individual tissue contractions on an arbitary scale of 0–1000 units (provided by the Bioengineering Department of St. Mary's Hospital Medical School). No attempt was made to study the individual components of the contractile response. In subsidiary experiments, the $CaCl_2$ concentration was varied over the range 1.25–5.0 mM with 30 min allowed for equilibration. Similarly, the frequency of tissue stimulation was varied over the range 0.2–6.4 Hz.

Animals and isolated tissue preparations

Sexually mature male mice (12–14 weeks, 20–25 g) of the following inbred strains were used in these studies: SWR/J, A2G and 129/Sv (OLAC); C57BL/6J, C5BL/10ScSn, A/J, DBA/2J, Balb/C and C3H (Animal Department, St. Mary's Hospital Medical School). F_1 mice of the cross 129/Sv (male) × SWR/J (female) and the backcross progeny of the mating 129/Sv (female) × [129/Sv × SWR/J] (male) were raised in the Department of Genetics and Biometry, University College, London.

All animals were killed by cervical dislocation, vasa deferentia immediately removed and stripped of adhering fat, connective tissue and blood vessels. Vasa were mounted separately in 2 ml organ baths under a resting tension of 500 mg. After an equilibration period of 30 min, the preparations were stimulated as described above for 2 min at 10 min intervals. When the response to stimulation was stable, concentration-response curves consisting of at least four different agonist concentrations were obtained, and,

in each instance, cumulative and single concentration additions of agonists were compared. Antagonists were allowed to equilibrate for 45 min before addition of agonist drugs.

Data analysis

The concentration of agonist producing 50% inhibition (I_{50}) of the twitch response was determined by log-probit least squares regression analysis of dose-response data. All I_{50} values quoted are based on at least eight tissues and four dose levels, except in the case of F_1 and backcross mice, where values quoted are means of those obtained from the two vasa taken from individual animals. Apparent dissociation constants (K_B) for antagonists were calculated by the dose-ratio method (Arunlakshana & Schild, 1959). For each antagonist, three concentration levels were employed (cimetidine 10, 30 and 100 μM; tiotidine 1, 3, and 10 μM). Where parameters were being compared between more than two strains, data were subjected to one-way analysis of variance; for a simple comparison of two strains Student's *t* test was employed.

Drugs

Histamine acid phosphate was obtained from BDH Chemicals PLC, Poole, dimaprit hydrochloride and cimetidine (free base) were the gift of Smith, Kline and French Laboratories PLC, Welwyn and tiotidine was the gift of ICI Pharmaceuticals PLC, Alderley Edge. Methysergide hydrogen-maleate was the gift of Sandoz Ltd, Basle, Switzerland. Haloperidol, mepyramine maleate, atropine sulphate and naloxone hydrochloride were obtained from the Pharmacy, St. Mary's Hospital. Yohimbine hydrochloride was obtained from Sigma Chemicals PLC, Poole. All other reagents used were Analar grade.

Results

Inter-strain variability survey

The variability of histamine I_{50} and cimetidine K_B values encountered in a study of vasa deferentia taken from 82 mice drawn from nine inbred strains is summarised in Figure 1 and Table 1. No differences of any significance were observed in the appearance, length or weight of the vasa between any of the strains or crosses used. The range in variability encountered in histamine I_{50} was 1.1–11.9 μM, and in cimetidine K_B 3.2–58 μM. One-way analysis of variance indicated that the degree of variability in both parameters was highly significant in relation to the variability within strains ($P<0.00001$ in both instances, Table 2). The antagonism of histamine by cimetidine was consistently competitive in all the strains studied, in no case was the slope of the Schild plot significantly different from unity (Table 1), and there was no statistical evidence for any inter-strain variability in this parameter. In no case did cimetidine alone have any effect on the twitch response. Inspection of the data in Table 1 and Figure 1 suggested the presence of two strain types; three sensitive (S) SWR, C57BL/10ScSn and A2G, and six insensitive (IS) C3H, A, C57BL/6, DBA/2 Balb/C and 129/Sv. Analysis of variance confirmed that each S strain was significantly different from the IS strains but not the other two S strains, irrespective of whether the comparison was based on histamine I_{50} or cimetidine K_B. Full concentration-response curves to histamine 0.1–300 μM in the absence and presence of cimetidine (10, 30 and 100 μM) are shown in Figure 2 for a representative S strain (SWR) and IS strain (C3H).

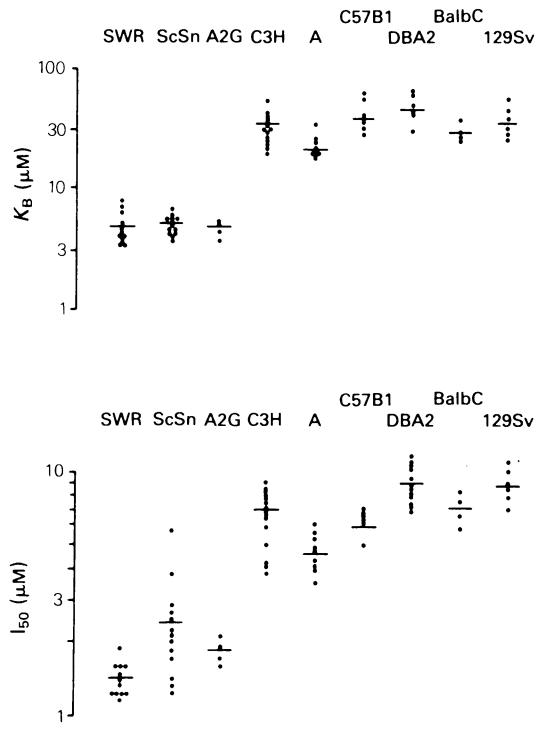

Figure 1 Strain distribution of cimetidine K_B (μM) and histamine I_{50} (μM) values in nine inbred mouse strains. Each point represents the mean value for parameters in two vasa deferentia taken from a single animal.

Table 1 Inter-strain variability in the murine vas deferens histamine H_2-receptor dose-response characteristics

Strain	n	*Histamine* I_{50} (μM)	*Cimetidine* K_B (μM)	*m*
SWR	12	1.4±0.2	6.0±1.7	0.92±0.19
C57BL/10ScSn	13	2.4±1.3	6.9±0.9	0.95±0.25
A2G	4	1.6±0.3	6.0±0.7	0.88±0.19
C3H	15	6.3±1.6	32.1±12.1	0.97±0.20
DBA/2	12*	10.0±0.8	42.5±5.0	0.89±0.18
A	9	4.9±1.0	21.4±5.4	1.09±0.17
C57BL/6	7	5.9±0.9	35.1±4.6	1.04±0.21
129/Sv	6	8.2±1.8	33.0±5.0	1.10±0.17
Balb/C	4	7.4±0.5	28.6±3.8	0.89±0.18

Mean ± s.d.; n = number of animals; both vasa being studied; m = slope of Schild plot ± 95% confidence limits).
* Histamine I_{50}, $n = 12$; cimetidine K_B, $n = 6$.

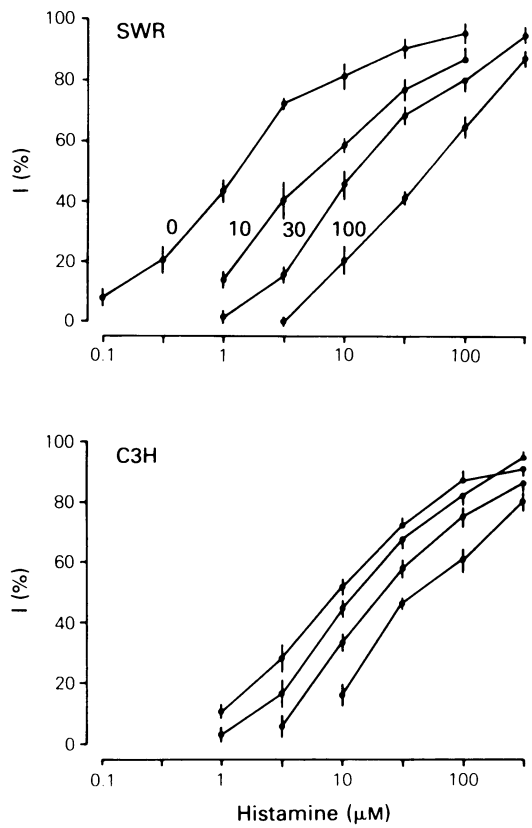

Figure 2 Concentration-response curves to histamine in the absence and presence of 10, 30 and 100 μM cimetidine (as indicated by numbers) in representative S (SWR) and IS (C3H) tissues. I (%) = percentage inhibition of the twitch response; all values are mean, $n = 12$ (SWR), $n = 15$ (C3H); s.d. shown by vertical lines.

Calcium dependence

The effects of variation in extracellular calcium concentration on patterns of inter-strain variability in histamine I_{50} and cimetidine K_B are summarised in Table 3. While raising the extracellular calcium concentration from 2.5 mM to 5 mM had only marginal effects on the twitch response, histamine I_{50} was significantly increased ($P < 0.05$) in all the four strains studied. On lowering the calcium concentration to 1.25 mM there was an approximately 50% reduction in the tension developed by the tissues on stimulation and in addition there was a significant reduction in histamine I_{50} ($P < 0.05$), except in the case of A2G, where no significant decrease was observed. In contrast, raising or lowering extracellular calcium concentration failed to elicit any significant alterations in cimetidine K_B values in any of the strains in which this aspect was studied.

Frequency-dependence

The effect of altering the frequency of tissue stimulation on the agonist potency of histamine is shown in Figure 3. This aspect was studied at two histamine dose levels (2 μM and 10 μM) and six frequencies of tissue stimulation (0.2, 0.4, 0.8, 1.6, 3.2, and 6.4 Hz) in two S (SWR and A2G) and two IS (C3H and DBA/2) strains. Although the tension developed on stimulation increased with increasing frequency, in no case was there a significant degree of frequency-dependence displayed by the response of any tissue from S or IS strains to histamine at either dose level.

Effects of non-histamine H_2-antagonists

The ability of non-histamine H_2-antagonists to inhibit the response to histamine (2 μM in two S strains,

Table 2 Analysis of variance in histamine I_{50} (μM) and cimetidine K_B (μM) values between and within nine inbred mouse strains

Source of variance	*Sum of squares*	*DF*	*S*	*F*
Histamine I_{50}				
Between strains	637.27	8	79.66	73.35
Within strains	79.28	73	1.09	($P<0.00001$)
Cimetidine K_B				
Between strains	27804.50	8	3475.50	84.90
Within strains	2746.30	67	41.05	($P<0.00001$)
Schild plot slope				
Between strains	0.39	8	0.05	1.11
Within strains	2.89	67	0.04	(NS)

DF = degrees of freedom; S = mean square variance estimate; F = variance ratio.

SWR and A2G, $n=4$; and 10 μM in two IS strains, C3H and DBA/2, $n=4$) was investigated using on separate occasions mepyramine (100 nM), atropine (100 nM), methysergide (1 μM), haloperidol (1 μM), yohimbine (100 nM) and naloxone (100 nM). With the exception of yohimbine which caused a slight (10%) potentiation, none had any effect on the twitch response alone. The response to histamine was unaffected in all instances by these antagonists at the concentrations used.

Dimaprit

The agonist potency of dimaprit (0.3–1000 μM) was studied in tissues from two S, SWR and A2G, and two IS, C3H and DBA/2, strains, $n=6$ for both. The I_{50} values obtained in the tissues from S mice were similar (overall: 4.6 ± 1.8 μM; SWR: 4.7 ± 1.3 μM; A2G: 4.4 ± 1.7 μM), although significantly higher I_{50} values were found for tissues from IS mice (overall: 48.7 ± 11.9 μM; C3H: 45.5 ± 12.0 μM; DBA/2:

Table 3 The influence of extracellular calcium concentration on histamine I_{50} (μM) and cimetidine K_B (μM) values from vasa taken from representative S and IS strains

Histamine I_{50}		*Calcium concentration*		
Strain	*S/IS*	5 mM	2.5 mM	1.25 mM
SWR	S	3.6 ± 0.4	1.4 ± 0.2	0.4 ± 0.1
A2G	S	7.8 ± 0.6	1.7 ± 0.6	1.6 ± 0.4
C3H	IS	18.8 ± 4.9	6.3 ± 1.6	3.2 ± 0.3
DBA/2	IS	12.9 ± 1.1	10.0 ± 0.8	1.9 ± 0.2

All parameters were significantly different ($P<0.05$) from their 2.5 mM $CaCl_2$ counterparts with the exception of A2G at 1.25 mM $CaCl_2$.

Cimetidine K_B		*Calcium concentration*		
Strain	*S/IS*	5 mM	2.5 mM	1.25 mM
SWR	S	5.3 ± 0.9	6.0 ± 1.7	5.7 ± 1.1
A2G	S	5.5 ± 1.1	6.0 ± 0.7	6.2 ± 0.5
C3H	IS	39.7 ± 9.8	32.1 ± 12.1	29.3 ± 7.7
DBA/2	IS	29.9 ± 16.1	42.5 ± 5.0	37.1 ± 8.9

($n=4$ for all experiments other than those for 2.5 mM $CaCl_2$, where n values are as for Table 1. Mean ± s.d.). No parameter values were significantly different from their 2.5 mM $CaCl_2$ counterparts.

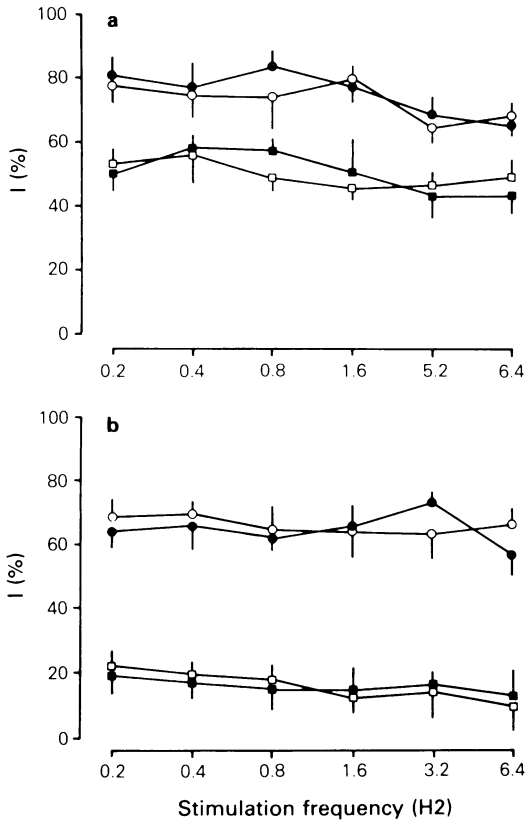

Figure 3 The inhibitory potency of histamine (10 μM, a, 2 μM b) at different frequencies of tissue stimulation. (● SWR; ○ A2G; □ C3H; ■ DBA/2). I (%) = percentage inhibition of twitch, all values are mean, $n = 4$ for all experiments; s.d. shown by vertical lines.

50.6 ± 12.8 μM). The mean ratio of the potency of histamine relative to dimaprit was thus approximately 2.5 in S mice, and approximately 6 in IS mice. Full concentration-response curves to histamine and dimaprit in tissues from SWR and DBA/2 mice are shown in Figure 4. Apart from differences encountered in I_{50} values for dimaprit, a second notable feature of this aspect of the study was the fact that the agonist actions were incomplete in IS mouse tissues, the drug being able to elicit no more than 75% inhibition of the twitch response at concentrations of 300–1000 μM. In contrast, the twitch response of tissues from S mice was completely inhibited by dimaprit at concentrations in excess of 100 μM.

Tiotidine

Tiotidine (1, 3 and 10 μM), while having no effect on the twitch response on its own, competitively antagonized the effects of histamine on tissues from two S strains, SWR and A2G, and two IS strains, C3H and DBA/2. The mean K_B value found with tissues from S mice was 189 ± 93 nM (SWR: 161 ± 86 nM, $n = 6$; A2G: 205 ± 90 nM, $n = 6$), and that in tissues from IS mice was 1007 ± 310 nM (C3H: 903 ± 182 nM; DBA/2: 1025 ± 206 nM, $n = 6$). In none of these studies was the slope of the Schild plot significantly different from unity. The S/IS strain differences with respect to sensitivity to tiotidine were thus of the same magnitude as those observed with cimetidine.

Strain breeding studies

The histamine I_{50} and cimetidine K_B values found in tissues from F_1 mice and from backcross progeny are

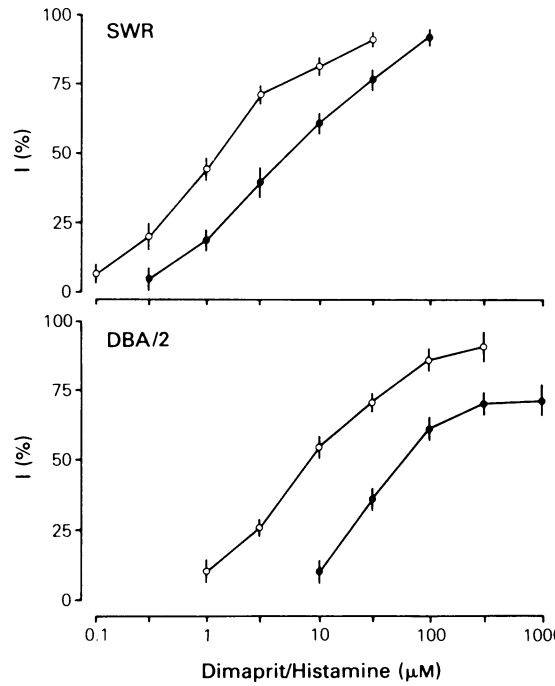

Figure 4 The relative inhibitory potencies of histamine (○) and dimaprit (●) in their inhibition of the twitch response of tissues from SWR and DBA/2 mice. I (%) = percentage inhibition of the twitch response; all values are mean, $n = 6$; s.d. shown by vertical lines.

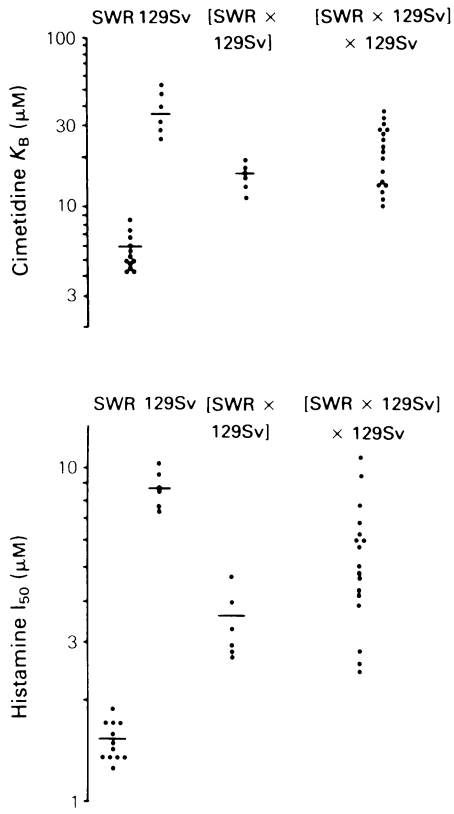

Figure 5 Distribution of histamine I_{50} and cimetidine K_B values in tissues taken from SWR × 129Sv F_1 mice and tissue taken from [SWR × 129Sv] × 129Sv backcross progeny; each point represents the mean value for parameters in two vasa deferentia taken from a single animal.

shown in Figure 5. In the F_1 mice ($n=6$) both the histamine I_{50} and cimetidine K_B values were intermediate with respect to those found in tissues from the parental strains (I_{50}: $3.6 \pm 0.9\,\mu$M, antilog mid-parent value $3.4\,\mu$M; K_B: $14.1 \pm 3.9\,\mu$M, antilog mid-parent value $13.2\,\mu$M), and both sets of parameters were significantly different ($P<0.05$) from those found in the parental strains. The progeny of the backcross displayed a wide range of values representative of those seen in tissues from both F_1 and parental 129/Sv mice. Of the seventeen backcross progeny studied, nine were F_1-like as judged by their position relative to the antilog mid-parent values for both parameters (expected for monogenetic inheritance: 8.5, $\chi^2=0.03$, $P<0.50$) and five were 129/Sv-like ($\chi^2=1.44$, $P<0.20$) However, three progeny could not be classified on this basis, being F_1-like for one parameter, and 129/Sv-like for the other.

Considering only histamine I_{50} values, the F_1-/129/Sv-like segregation was 11:6 ($\chi=1.47$, $P<0.20$), and considering only cimetidine K_B values, the segregation was 10:7 ($\chi^2=0.53$, $P<0.40$). The overall correlation between histamine I_{50} and cimetidine K_B values in the backcross progeny was significant ($r=0.78$, $P<0.0013$); it is clear from these data, however, that the pattern of histamine and cimetidine sensitivities found in these progeny does not allow any definite conclusions to be drawn as to the exact mode of inheritance of the S/IS phenotype.

Discussion

A survey of the ability of histamine to inhibit the twitch response of the isolated vas deferens of the mouse to electrical field stimulation, and also of the ability of cimetidine to antagonize this response, has revealed eight to ten fold variation in both parameters between nine inbred mouse strains. Evidence has been presented to support the view that this variability represents authentic qualitative genetic modification of the histamine H_2-receptor present in this tissue.

The possibility that histamine or cimetidine may be interacting non-specifically with other receptor systems, such as those responsive to noradrenaline, 5-hydroxytryptamine, dopamine, acetylcholine or morphine, in some strains but not in others, has been ruled out by the use of appropriate selective antagonists. Furthermore, the possibility that vasa from some strains but not others contain H_1-receptors as well as H_2-receptors, as has been demonstrated for other species (Vohra, 1981) has been excluded by the failure of mepyramine to modify the response of tissue from any strain to the effects of histamine. It would also appear unlikely that the strain differences arise from variable tissue susceptibility to tachyphylaxis or desensitisation, since that same patterns of sensitivity to histamine were found regardless of whether concentration-response curves were constructed cumulatively or in a single concentration fashion.

The effects of varying extracellular calcium and frequency of tissue stimulation on the pattern of strain variability were studied for two reasons. First, the evidence currently available would suggest that histamine exerts at least some of its inhibitory effects on the mouse vas deferens by restricting calcium influx into postsynaptic tissue (Marshall, 1978; Vohra, 1979); thus, it is possible that strain differences in histamine sensitivity could arise through

differences in either calcium utilisation by smooth muscle or in membrane components other than the H_2-receptor responsible for modifying rates of calcium entry. Secondly, it is possible that in some strains but not in others, pre- as well as postsynaptic H_2-receptors are present which may exert an inhibitory effect by reducing neurotransmitter output, in the manner of clonidine (Starke *et al.*, 1975) or morphine (Henderson & Hughes, 1976). Extracellular calcium concentration is clearly a potent modifier of histamine sensitivity, as found here, and previously reported by Vohra (1979). However, this variable did not affect the strain distribution patterns of the antagonist potency of cimetidine, clearly implicating the H_2-receptor itself as the site of genetic modification. Interestingly, the shifts in histamine I_{50} elicited by varying extracellular calcium concentration showed some differences within S and IS strains. Most notably, in tissues from A2G mice, reduction of extracellular calcium from 2.5 M to 1.25 M failed to alter significantly histamine I_{50} even though this reduction elicited a two to five fold increase in histamine potency in the other three strains studied, suggesting that variable susceptibility to changes in calcium concentration may also be a strain-dependent characteristic, independent of the variation in H_2-receptors which is of immediate interest in the present study.

The failure of changes in rates of tissue stimulation to alter strain distribution patterns of histamine sensitivity makes it unlikely that the strain differences arise from variable pre- or postsynaptic location of the H_2-receptor. Effects mediated by presynaptic receptors are classically frequency-dependent (Marshall *et al.*, 1979), and none of the responses of tissues from S or IS strains displayed such a characteristic. Confirmation of this supposition strictly requires measurement of transmitter release in representative S and IS strains.

If the data presented do indeed reflect genetic modification of the histamine H_2-receptor in the mouse vas deferens, this modification may be qualitative, i.e. in receptor structure or in its incorporation into the cell membrane, or it may be quantitative, i.e. in receptor number. Whilst these possibilities are clearly not mutually exclusive, the fact that the affinity constants of two histamine H_2-antagonists of considerably differing potency showed significant strain variation and that the relative potencies of histamine and dimaprit displayed parallel strain differences would strongly suggest qualititative modification. There is currently considerable controversy as to whether or not the histamine H_2-receptor is a homogeneous entity between different tissues and different species. The existence of H_2-receptor sub-types has been postulated (Bertaccini & Coruzzi, 1981), though this subdivision is far from widely accepted. If, however, such a subdivision were justified, it is possible that the present findings reflect strain differences in relative proportions of the putative H_2-receptor sub-types.

We are currently evaluating the properties of [^{3}H]-tiotidine and [^{3}H]-histamine as H_2-receptor ligands in the mouse vas deferens; it is hoped that radioligand receptor binding studies will, first, improve discrimination of S and IS strains and, secondly, provide evidence as to whether or not differences in receptor number contribute to the sensitivity differences between strains.

When inbred strains of mice fall into two classes with respect to any characteristic it is usually found that a single gene is responsible for the difference between the classes (Lush, 1981). With the nine strains used in this work, the cimetidine K_B values in particular appeared to fall very convincingly into two classes, S and IS. If one gene is responsible for the difference between the S and IS phenotypes than the backcross progeny should segregate in approximately equal numbers into two groups, one 129/Sv- like and one F_1-like. However, there was no trace of the expected bimodality in the backcross data. The results of backcrossing, therefore, provided no evidence to support the premise that a single gene mode of inheritance of histamine-sensitivity was in operation and that, as a consequence, the possibility that polygenic mechanisms are involved perhaps becomes more likely.

The wider biological significance of these findings remains to be evaluated. The principal application of drugs interacting with histamine H_2-receptors in man is the use of H_2-antagonists in the regulation of gastric acid secretion. There are now a number of reports in the literature of 'non-responders' to cimetidine treatment (Hasan & Sircus, 1980; Hunt, 1981; Bardham, 1981). Interestingly, such differences in responsiveness to cimetidine do not appear to have a pharmacokinetic basis; the rates of absorption and plasma levels of cimetidine were the same in responding and non-responding groups of patients (Hunt, 1981) but any link between those findings and the present investigation must be purely speculative.

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