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THE EFFECTS OF HISTAMINE H_2 RECEPTOR ANTAGONISTS ON ANDROGEN ACTION *IN VIVO* AND DIHYDROTESTOSTERONE BINDING TO THE RAT PROSTATE ANDROGEN RECEPTOR *IN VITRO*

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Abstract—Several histamine H_2 receptor antagonists have been tested for antiandrogenic activity by determining their effects on accessory sex organ weights in castrate testosterone propionate (TP) treated rats and on [^{3}H]dihydrotestosterone (DHT) binding to the androgen receptor of the rat ventral prostate *in vitro*.

When given in high doses cimetidine and metiamide possessed antiandrogenic activity whereas the other H_2 receptor antagonists SK&F 92456, SK&F 92994, SK&F 92629 and SK&F 93479 did not. Cimetidine, metiamide and SK&F 92456 inhibited [^{3}H]DHT binding to rat ventral prostate androgen receptor *in vitro* whereas SK&F 92629 and SK&F 93479 did not. SK&F 92994 affects DHT binding only slightly.

Of the compounds that are similar to cimetidine in their potency as H_2 receptor antagonists only metiamide was antiandrogenic. However SK&F 92994 and SK&F 93479 are not antiandrogenic despite being more potent than cimetidine as H_2 antagonists. It is concluded that the antiandrogenicity of cimetidine and metiamide was not related to their activity as histamine H_2 receptor antagonists.

Toxicological studies with the histamine H_2 receptor antagonist cimetidine (Tagamet: *N*″-cyano-*N*-methyl-*N*′-{2-[(5-methylimidazol-4-yl)methylthio]-ethyl} guanidine) demonstrated that when given in high doses to intact rats, growth of the prostate and seminal vesicles was reduced [1]. This could have been due to the antagonism of androgen action at target organ sites (antiandrogenicity); alternatively it could have resulted from an inhibition of androgen synthesis either directly or indirectly by way of reduced gonadotrophin levels. Evidence that cimetidine possessed antiandrogenic activity came from Saunders and co-workers [2] who found that this compound did not affect plasma LH or testosterone levels when given to intact rats but directly antagonized the effects of exogenous testosterone on the prostate and seminal vesicles of castrate rats. These workers found that 320 mg/kg/day was the minimum dose of cimetidine needed to show antiandrogenic activity (as judged by the weights of both accessory sex organs). However, Winters, Banks and Loriaux later reported that cimetidine showed antiandrogenic activity when given at 50 mg/kg/day to castrate rats [3].

The antiandrogenic activity of cimetidine could result from a blockade at any stage of the mechanism of androgen action. In the rat prostate, testosterone is first converted to the more active androgen, 5α-dihydrotestosterone (DHT), by 5α reductase.† DHT then binds to a cytosol receptor. This is translocated to the nucleus where it binds to nuclear acceptor sites resulting in the biological response (for review see [4]). Whilst there is no information as to whether 5α reductase is affected by cimetidine, this compound has been found to inhibit the binding of DHT to the androgen receptor of the mouse kidney [5] and rat ventral prostate [3]. In the latter study however there was no effect of the H_2 receptor antagonist metiamide (*N*-methyl-*N*′-{2-[(5-methylimidazol-4-yl)-methylthio]ethyl} thiourea) on DHT binding although this compound has been found to inhibit the action of exogenous testosterone on immature intact rats [6].

There is therefore some discrepancy concerning the potency of cimetidine as an antiandrogen and some question as to whether other H_2 antagonists are antiandrogenic. We have re-examined the potency of cimetidine as an antiandrogen, the mechanism of this effect and also the relationship between H_2 receptor antagonist activity and antiandrogenicity. The effects of cimetidine have been compared with the antiandrogen cyproterone acetate and with the aldosterone antagonist spironolactone which also has antiandrogenic properties [7, 8].

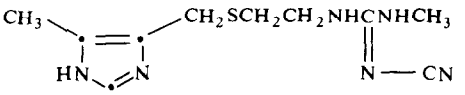
MATERIALS AND METHODS

Materials

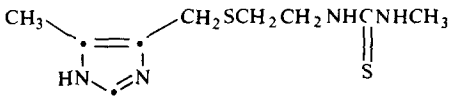
5α-Dihydro-[1,2,4,5,6,7-^{3}H]testosterone was obtained from the Radiochemical Centre (Amersham, U.K.). Silastic tubing (1 inch × 0.062 inches i.d.; 0.125 inches o.d.) was obtained from Dow Corning Corporation (Midland, MI). Cyproterone acetate was kindly donated by Schering Chemicals Ltd.

* To whom all correspondence should be sent.
† NADPH Δ^4-3-ketosteroid 5α reductase.

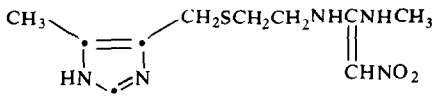
Cimetidine



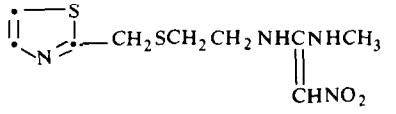
Metiamide



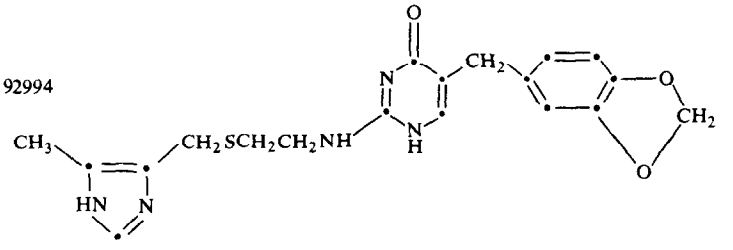
SK & F 92456



SK & F 92629



SK & F 92994



SK & F 93479

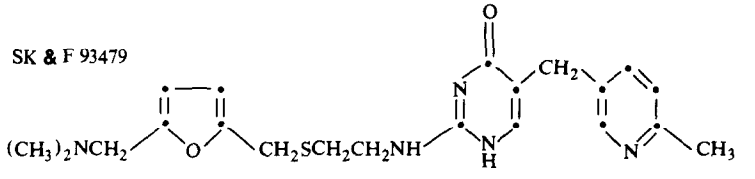


Fig. 1. Structures of H_2 receptor antagonists.

(Berlin, West Germany). Steroids and mercaptoethanol were obtained from Sigma (London) Chemical Co. (Poole, U.K.); arachis oil from Hopkin and Williams Ltd. (Chadwell Health, U.K.); dextran T70 from Pharmacia Ltd. (Uppsala, Sweden); Pico-Fluor 15 scintillation solution from Packard Instruments (Reading, U.K.); Immobilon (etorphine hydrochloride) anaesthetic from Reckitt and Colman Ltd. (Hull, U.K.); and all other chemicals from BDH Ltd. (Poole, U.K.).

The histamine H_2 receptor antagonists used on this study were synthesized by the Department of Medicinal Chemistry of Smith Kline and French Research Ltd. Their structures are shown in Fig. 1 and further information concerning these compounds can be obtained from the references given in Table 3.

In vivo *antiandrogen bioassay*

Male Wistar rats aged 21–26 days, were obtained from the colony of the Research Institute, Smith Kline and French Research Ltd., and were allowed food and water *ad libitum*. Animals were anaesthetized with etorphine hydrochloride, 0.05 ml intraperitoneally (i.p.) and then castrated, using the scrotal route following ligation of the spermatic cord. Treatment with steroids and histamine H_2 receptor antagonists commenced 24 hr after castration and continued for 7 days. TP and DHT were administered subcutaneously (s.c.) once daily in 0.1 ml/100 g body wt of arachis oil. In one experiment testosterone was released from a silastic capsule implanted s.c. on the day of castration. H_2 receptor antagonists were dissolved in the minimum volume of N HCl, the solution adjusted to pH 6.0 with NaOH and

diluted with distilled water unless otherwise stated in the figures. These compounds were administered orally at a volume of 0.5 ml/100 g body wt or in one experiment i.p. at a volume of 0.1 ml/100 g body wt. Spironolactone and cyproterone acetate were administered orally in 0.5% sodium carboxymethylcellulose (0.5 ml/100 g body wt). H_2 receptor antagonists, spironolactone and cyproterone acetate were given once daily for two days, while for the remaining five days half the daily dose was given at 0900 hours and the other half at 1700 hours. Cimetidine has been shown to produce the same reduction in accessory sex organ weights in castrate TP treated rats using this protocol compared with that found when rats were doses twice daily for seven days (data not shown). Twenty-four hours after the last dose of TP, the rats were killed by cervical dislocation. The ventral prostates and seminal vesicles with coagulating gland were dissected out and weighed. The body weights were also recorded. Statistical analyses were carried out using Student's *t* test.

In vitro *dihydrotestosterone receptor binding assay*

Preparation of cytosol. Male Wistar rats of 250–300 g body wt were castrated 24 hr prior to assay. On the day of assay, animals were killed by cervical dislocation. The ventral prostate was dissected out and immediately placed in homogenization medium (0.01 M sodium phosphate, 0.25 M sucrose, 4mM mercaptoethanol, pH 7.4) at 0–4° at which temperature all subsequent steps were carried out. Eight prostates were pooled and homogenized by hand in 10 ml of homogenization medium using a glass homogenizer. The homogenate was then centrifuged for 1 hr at 105,000 *g*. After centrifugation, the supernatant was removed and diluted to 20 ml with buffer (0.01 M sodium phosphate–0.25 M sucrose) to give a solution with a protein concentration of 1.0–1.5 mg/ml as measured by the method of Hartree [9].

Assay. Diluted cytosol (0.25 ml) was added to 0.1 ml of [^{3}H]DHT (approximately 0.05 μCi, 500 fmole per tube, final concentration 1.1×10^{-9} M) and 0.1 ml of buffer containing unlabelled DHT or competitor. The assay mixture was incubated at 0–4° for 2 hr after which time 0.5 ml of dextran coated charcoal (1 g activated charcoal, 0.1 g dextran T70 in 100 ml of 0.01 M sodium phosphate buffer, pH 7.6) was added. The assay mixture was then incubated for a further 25 min and then centrifuged at 650 *g* for 15 min at 0–4°. Aliquots (0.5 ml) of the supernatant were then mixed with 10 ml of Pico-Fluor 15 scintillation solution and counted on a Nuclear Enterprises liquid scintillation counter.

RESULTS

In vivo *assay*

Figure 2 shows the effects of different doses of cimetidine when given to immature rats receiving a fixed (0.2 mg/kg/day) dose of testosterone propionate (TP) s.c. Cimetidine given orally at 950 mg/kg/day for 7 days reduced the response of both the prostate and seminal vesicle weights to TP, whereas at 475 mg/kg/day only the seminal vesicle weights

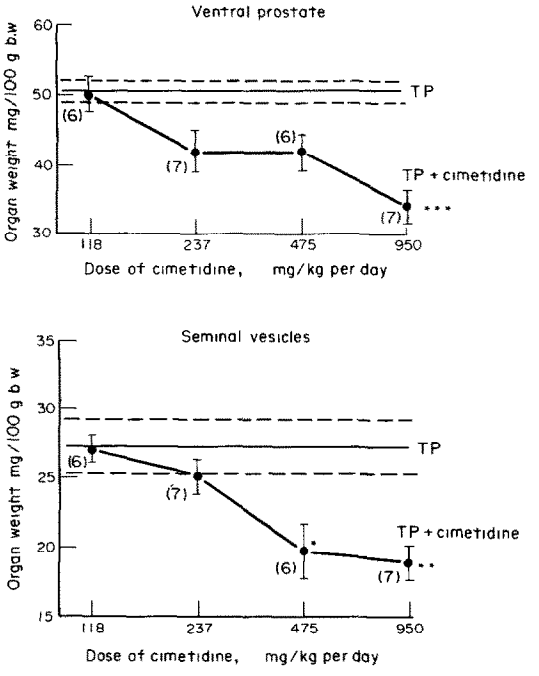


Fig. 2. Accessory sex organ weights in 21–26 day old castrate rats treated with testosterone propionate (TP, 0.2 mg/kg) and varying doses of cimetidine orally for 7 days (mean ± S.E.M., number of observations in parentheses). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, when compared to TP alone.

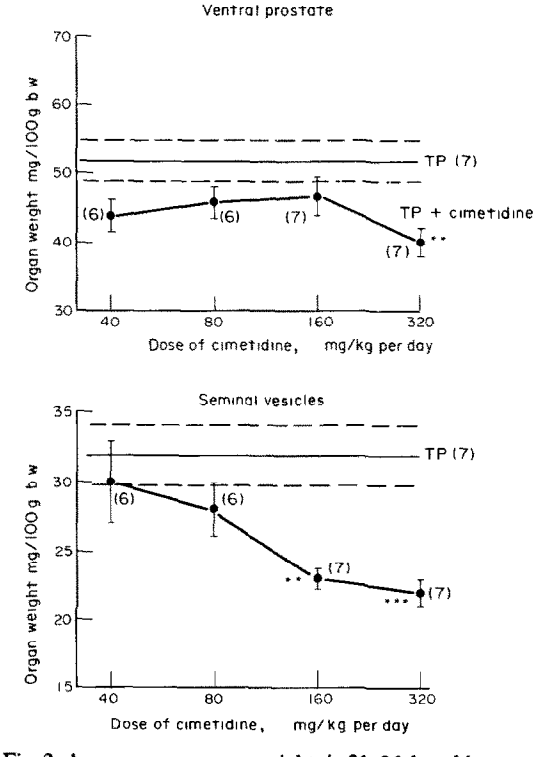


Fig. 3. Accessory sex organ weights in 21–26 day old castrate rats treated with testosterone propionate (TP, 0.2 mg/kg) and varying doses of cimetidine intraperitoneally (0.2 ml/100 g) for 7 days (mean ± S.E.M., number of observations in parentheses). ** $P < 0.01$, *** $P < 0.001$, when compared to TP alone.

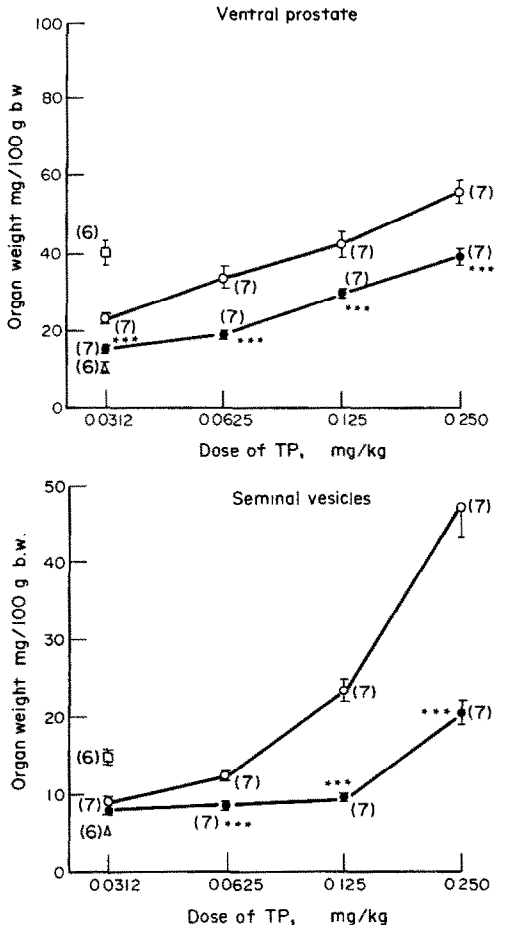


Fig. 4. The effects of cimetidine (950 mg/kg/day) on accessory sex organ weights in 21–26 day old castrate rats given testosterone propionate (TP) daily for 7 days (mean ± S.E.M., with the number of observations in parentheses). ○—○, TP alone; ●—● TP + cimetidine; □, intact; △, castrate. *** $P < 0.001$, when compared to TP alone.

were significantly reduced. No antiandrogenic activity was demonstrable at oral doses below 475 mg/kg/day. When cimetidine was given i.p. for 7 days prostatic weights were not affected at doses below 320 mg/kg/day or seminal vesicle weights below 160 mg/kg/day (Fig. 3).

Figure 4 shows the effects of cimetidine (950 mg/kg/day) on the dose–response curve to TP. There was a downward shift. Cimetidine reduced the response of the prostate to each dose of TP used and the response of the seminal vesicles to 0.0625, 0.125 and 0.25 mg/kg of TP.

To determine if the antiandrogenic activity of cimetidine might be a consequency of decreased 5α reductase activity or a block in later stages of androgen action, one assay was carried out using DHT as the agonist. Cimetidine reduced the response of the prostate to each dose of DHT given (Fig. 5). The seminal vesicles were less sensitive to the stimulatory effects of DHT than was the prostate so that only the highest dose of DHT (0.25 mg/kg/day) significantly increased seminal vesicle weight above that of castrated controls. This response was totally abolished by cimetidine.

Because of the discrepancy between the dose of cimetidine that showed antiandrogenic activity in our assay and that reported by Winters, Banks and Loriaux [3] we have repeated their experiment using the materials and also the methods exactly as described by these workers. Table 1 shows that cimetidine given i.p. twice daily for 7 days at a total daily dose of 50 mg/kg failed to affect the response of the accessory sex organs to testosterone released from silastic implants. In this experiment the response of the prostate to testosterone was generally similar to that found previously [3] whereas the response of the seminal vesicles was slightly greater.

Several other histamine H_2 receptor antagonists have been examined for antiandrogenic activity. Figure 6 shows that metiamide (SK&F 92058) at 920 mg/kg/day, a dose equimolar to the highest dose of cimetidine used, partially inhibited the response of the prostate to all doses of TP used and almost completely inhibited the response of the seminal vesicles to TP.

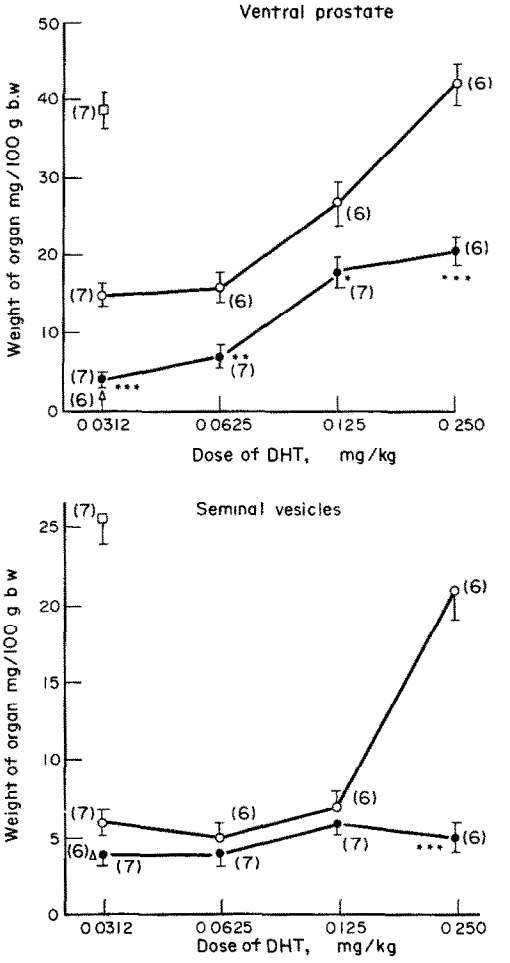


Fig. 5. The effects of cimetidine (950 mg/kg/day) on accessory sex organ weights in 21–26 day old castrate rats given varying doses of dihydrotestosterone (DHT) for 7 days (mean ± S.E.M., with the number of observations in parentheses). ○—○, DHT alone; ●—●, DHT + cimetidine; □, intact; △, castrate. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, when compared to DHT alone.

Table 1. The effects of cimetidine (50 mg/kg/day) given intraperitoneally on accessory sex organ weights in adult castrate rats receiving testosterone from silastic implants

Treatment	Weight of ventral prostate (mg/100 g body wt)	Weight of seminal vesicles (mg/100 g body wt)
Intact control	98 ± 7 (6)	270 ± 13 (6)
Castrate control	34 ± 3 (6)	67 ± 9 (6)
Castrate + testosterone	116 ± 7 (6)	434 ± 34 (6)
Castrate + testosterone + cimetidine	128 ± 5 (6)	416 ± 25 (6)

Results are expressed as means ± S.E.M., with the number of observations in parentheses.

The effects of four other H_2 antagonists on accessory sex organ growth stimulated by a fixed (0.2 mg/kg) dose of TP as shown in Table 2. Neither SK&F 92456 (1005 mg/kg/day), SK&F 92994 (1779 mg/kg/day), SK&F 92629 (1030 mg/kg/day) or SK&F 93479 (1950 mg/kg/day) showed any antiandrogenic activity. These doses are equivalent on a molar basis to the highest dose of cimetidine used.

For comparison, the aldosterone antagonist spironolactone was also tested for antiandrogenic activity against a fixed (0.2 mg/kg/day) dose of TP. Prostatic weights were significantly reduced with doses as low as 25 mg/kg/day (Fig. 7). In a similar assay the classical antiandrogen cyproterone acetate reduced TP stimulated accessory sex organ growth when given at only 3.12 mg/kg/day (Fig. 8).

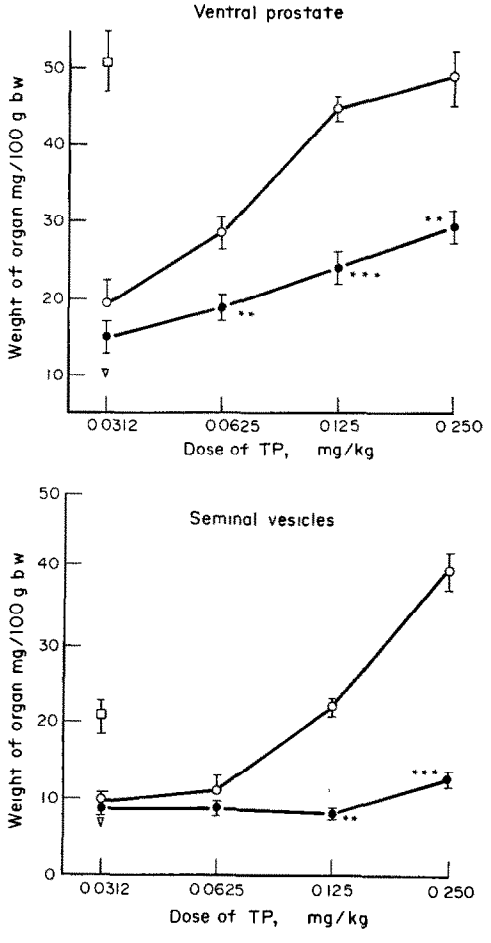


Fig. 6. The effects of metiamide (920 mg/kg/day) on accessory sex organ weights in 21–26 day old castrate rats given varying doses of testosterone propionate (TP) for 7 days (mean ± S.E.M., number of observations = 6). ○—○, TP alone; ●—●, TP + metiamide; □, intact; △, castrate. ** $P < 0.01$, *** $P < 0.001$, when compared to TP alone.

In vitro *assay*

The inhibition of DHT stimulated accessory sex organ growth by cimetidine suggested that this compound inhibited androgen action at stages beyond the formation of DHT. The effects of H_2 receptor antagonists on [^{3}H]DHT binding to the androgen receptor of the rat ventral prostate were therefore examined and compared with those found with cyproterone acetate and spironolactone. Figures 9a and b show the effects of these compounds on the binding of a fixed concentration (1.1×10^{-9} M) of [^{3}H]DHT. Assuming all specific binding sites to be saturated by a 200 fold molar excess of unlabelled DHT then approximately 55% of the total binding in Figure 8a and 65% in Figure 8b was specific. Cyproterone acetate, spironolactone, cimetidine, metiamide and SK&F 92456 all reduced the specific binding of [^{3}H]DHT. From the displacement curves the concentration of each inhibitor required to reduce the specific binding by 50% (an approximate IC_{50}) were as follows: DHT, 1.6×10^{-9} M; cimetidine, 8.0×10^{-6} M; spironolactone, 1.7×10^{-8} M; cyproterone acetate, 8.0×10^{-9} M; metiamide, 2.5×10^{-5}; SK&F 92456, 4.5×10^{-5}. SK&F 92994 caused only a slight reduction in [^{3}H]DHT binding (25% reduction in specific binding, 15% reduction in total binding). SK&F 92629 and SK&F 93479 failed to affect specific [^{3}H]DHT binding (SK&F 92629 data not shown).

DISCUSSION

The present study had three basic objectives: to compare the antiandrogenic activity of cimetidine with that of other known antiandrogens; to explore the relationship between antiandrogenic activity and H_2 receptor antagonism using H_2 antagonists of dif-

Table 2. The effects of SK&F 92456, SK&F 92994*, SK&F 92629 and SK&F 93479 on accessory sex organ weights in castrate rats given testosterone propionate† daily for 7 days

Treatment	Weight of ventral prostate (mg/100 g body wt)	Weight of seminal vesicles (mg/100 g body wt)
TP	57 ± 2 (6)	37 ± 2 (6)
TP + SK&F 92456 (1005 mg/kg/day)	56 ± 3 (6)	40 ± 3 (6)
TP	55 ± 4 (6)	39 ± 2 (6)
TP + SK&F 92994 (1779 mg/kg/day)	57 ± 4 (5)	46 ± 2 (5)
TP	54 ± 1 (7)	47 ± 2 (7)
TP + SK&F 92629 (1030 mg/kg/day)	51 ± 3 (6)	42 ± 4 (6)
TP	53 ± 3 (6)	36 ± 2 (6)
TP + SK&F 93479 (1950 mg/kg/day)	55 ± 1 (6)	33 ± 0.8 (6)

* 2 ml/100 g body wt.
† 0.2 mg/kg/day.
Results are means ± S.E.M., with the number of observations in parentheses.

ferent chemical structures; and finally to investigate the mechanism of action of cimetidine as an antiandrogen.

Our results have demonstrated that although cimetidine possessed antiandrogenic activity, as has been shown previously [2, 3], this effect was extremely weak. When calculated on a molar basis, the dose of cimetidine needed to inhibit the action of TP on both accessory sex organs was some 543 times that of the classical antiandrogen cyproterone acetate and sixteen times that of spironolactone when tested under identical conditions. We have been unable to confirm the findings of Winters, Banks and

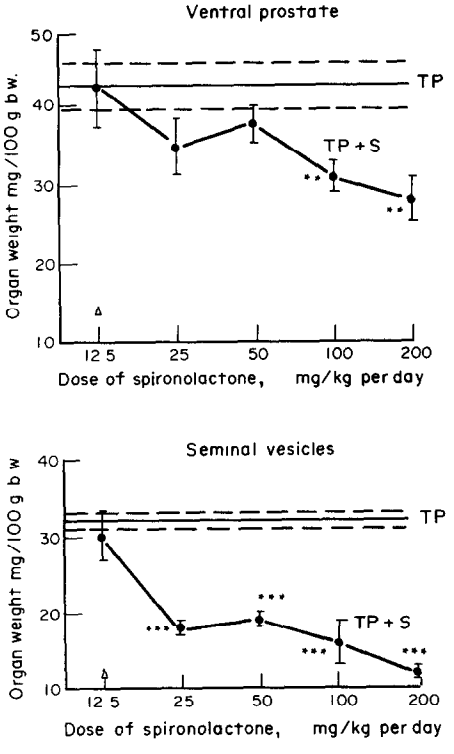


Fig. 7. Accessory sex organ weights in 21–26 day old castrate rats treated with testosterone propionate (TP, 0.2 mg/kg) and varying doses of spironolactone (S) orally for 7 days (mean ± S.E.M., number of observations = 6). ** $P < 0.01$, *** $P < 0.001$, compared with TP alone.

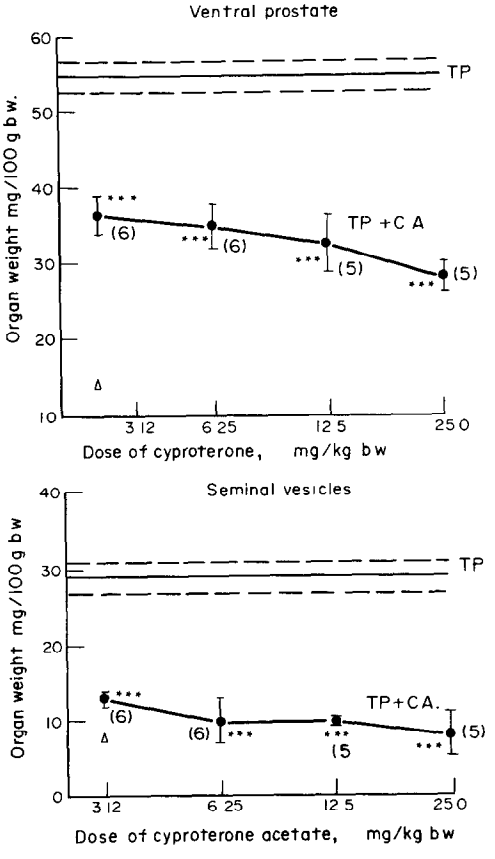


Fig. 8. Accessory sex organ weights in 21–26 day old castrate rats treated with testosterone propionate (TP, 0.2 mg/kg) and varying doses of cyproterone acetate (C.A.) for 7 days (mean ± S.E.M., number of observations in parentheses). ** $P < 0.001$, when compared with TP alone.

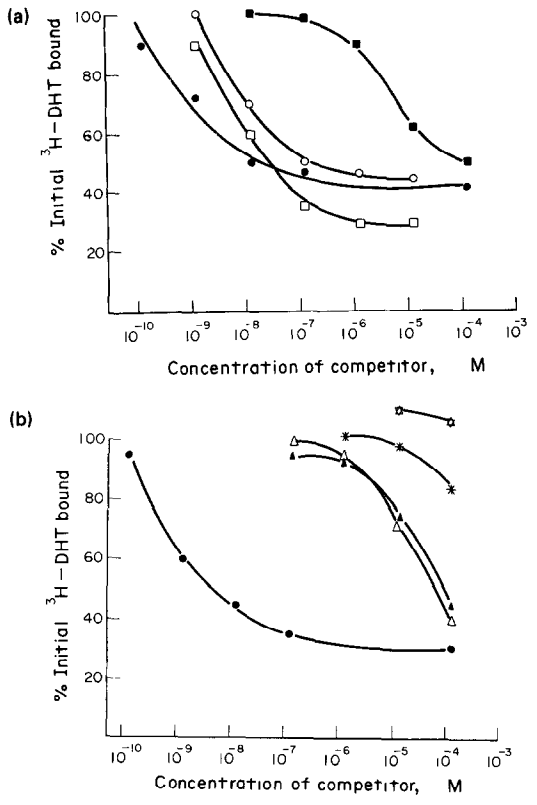


Fig. 9a and b. Displacement of bound [^{3}H]dihydrotestosterone (DHT) from androgen receptors in rat ventral prostate by unlabelled DHT, spironolactone, cyproterone acetate and histamine H_2 receptor antagonists. ●—●, DHT; ■—■, cimetidine; ○—○, spironolactone; □—□, cyproterone acetate, *—* SK&F 92994, ▲—▲ SK&F 92456, △—△ metiamide, ☆—☆ SK&F 93479.

Loriaux [3] that cimetidine was antiandrogenic when given at a dose of 50 mg/kg/day i.p. even though identical conditions were used. The finding that cimetidine caused a roughly parallel shift to the right in the dose–response curve to TP is consistent with competitive antagonism of androgen action.

The observation that cimetidine inhibited the effects of TP on accessory sex organ weights, albeit weakly, could imply that the histamine H_2 receptor is involved in some way with the action of TP. In order to explore this possibility we have compared the antiandrogenic activity of cimetidine with that of four other H_2 receptor antagonists of varying structures and potencies. Three of these compounds, metiamide, SK&F 92456 and SK&F 92629 are not significantly different from cimetidine in their H_2 antagonist potency (measured as their dissociation constants at the guinea pig right atrium H_2 receptor, see Table 3). However only metiamide showed antiandrogenic activity *in vivo* whereas SK&F 92456 and SK&F 92629 did not. SK&F 92994 and SK&F 93479 also failed to show antiandrogenic activity *in vivo* despite being significantly more active than cimetidine as H_2 receptor antagonists (Table 3). It is clear that antiandrogenic activity is not related to H_2 antagonist activity and therefore the inhibition of the effects of TP by cimetidine and metiamide does not involve histamine H_2 receptors.

Another explanation could be that cimetidine had inhibited the action of TP by preventing its activation by reduction to DHT. However the observation that the androgenic activity of DHT was also inhibited by cimetidine (Fig. 4) eliminated this possibility and placed the site of action of cimetidine at a stage beyond DHT formation. This was confirmed by *in vitro* studies (Figs. 9a and b) which showed that cimetidine and metiamide inhibited specific DHT binding by the prostatic androgen receptor. The antiandrogenic activity of these compounds appears therefore to be a consequence of reduced DHT binding to target organ receptors, a mechanism in common with classical antiandrogens [10]. The *in vitro* results obtained with cimetidine are in broad agreement with those of Funder and Mercer [5] and Winters, Banks and Loriaux [3]. The latter workers however did not find any effect of metiamide on DHT binding which is at variance with our findings. Comparisons of IC_{50} values show that as an inhibitor of DHT binding cimetidine is 5000 times weaker than DHT, 1000 times weaker than cyproterone

Table 3. Comparison of H_2 antagonist potency and antiandrogenic activity of H_2 receptor antagonists

Compound	Antiandrogenic activity *in vivo*	*Ki* (μM)	K_B (μM with 95% confidence limits)
Cimetidine	+	2.5	0.79 (0.68–0.92)
Metiamide	+	8.0	0.92 (0.74–1.15)
SK&F 92456†	–	14.0	1.4 (1.2–1.6)
SK&F 92629†	–		1.07 (0.65–1.78)
SK&F 92994	–	>600	0.2*
SK&F 93479	–		0.017 (0.049–0.0033)

* An estimated value from dose ratio = 2 (see Ref. 16).

† G. J. Durant, J. C. Emmett, C. R. Ganellin and H. D. Prain, *British Patent* 1, 421, 792 (1976). See also Smith *et al.* [21].

Ki, inhibition constant at androgen receptor (see Ref. 20).

K_B, apparent dissociation constants at the H_2 receptor determined *in vitro* against histamine stimulation of the rate of beating of the guinea pig right atrium. Results from Durant *et al.* [15], Blakemore *et al.* [16, 19], Black *et al.* [17], and Brimblecombe *et al.* [18].

(+), Presence.

(–), Absence.

acetate and 470 times weaker than spironolactone. From these figures it appears that the antiandrogenic activity of cimetidine compared to spironolactone is less when assessed *in vitro* (inhibition of [^{3}H]DHT binding) than when assessed *in vivo* (minimum dose required to depress prostatic and seminal vesicle weights in TP treated, castrated rats). Pharmacokinetic differences between these compounds probably contribute to this discrepancy; the serum half life of spironolactone in the rat (4–5 min, see [11] is considerably shorter than that of cimetidine (2 hr see [12]).

The antiandrogenic activity of cimetidine and metiamide *in vivo* correlated with their ability to bind to the androgen receptor *in vitro*. However, SK&F 92456 did not inhibit the action of TP *in vivo* but bound to the androgen receptor, although with an affinity six times less than cimetidine. It is known that after oral dosing SK&F 92456 did not achieve the same blood concentration as did an equimolar dose of cimetidine [13] which may account for this discrepancy.

It is of interest that SK&F 92629 and SK&F 93479, which do not possess the 5-methylimidazolyl ring do not inhibit androgen binding. Hence the antiandrogenic activity shown by some H_2 receptor antagonists may be associated with the presence of this ring structure as suggested by Yellin *et al.* [14]. However, possession of the 5-methylimidazolyl ring is not a sufficient criterion for activity since SK&F 92994 which is an isocytosine analogue of cimetidine is much less active as an inhibitor of binding.

It is concluded that cimetidine and metiamide possess some antiandrogenic activity but only when given in high doses. This appears to be due to an inhibition of DHT binding to the prostatic androgen receptor. However, antiandrogenic activity is not related to H_2 receptor antagonist activity.

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