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ARE THERE DISTINCT DIHYDROTESTOSTERONE AND TESTOSTERONE RECEPTORS IN BRAIN?

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SUMMARY

Investigations of cytosol receptor binding of androgen in brain have been carried out using either labelled testosterone or labelled dihydrotestosterone as ligand. The affinities of the binding of the ligands are in the same order of magnitude, competition shows that unlabelled excess of one can prevent binding of the other and both the testosterone and dihydrotestosterone high affinity binding moieties are rather uniformly distributed in brain. However, it is possible that these observations might conceal the existence of selective binding of one or other of the two ligands.

Cytosols were prepared from brain and ventral prostate in adult male rats 2 days and 3 days after orchidectomy and from brain in intact adult females. In each cytosol the capacities and affinities of saturable, high affinity binding of both [^{3}H]-dihydrotestosterone were measured by Scatchard plot analysis. Twenty-one pairs of observations were made with brains from females and although the extreme differences in ligand binding capacities between pairs was about 5-fold, there was near perfect correspondence in estimates of the saturation capacities for the two ligands. Similarly, while the abundance of binding sites fell between the 2nd and 3rd day after orchidectomy (by 50% in brain and 80% in ventral prostate) the ratios of the saturation capacities were not significantly different from 1.0.

INTRODUCTION

The advance in knowledge that has dominated the study of androgen action in the past ten years is the discovery that in many androgen sensitive tissues testosterone is converted to other substances through whose action the effects of testosterone secretion or injection are elicited. Where the metabolic product has a much higher affinity for the relevant receptor as in the case of the conversion of testosterone to oestradiol, this can be seen clearly as the selective generation of the active substance in a limited population of cells. However, in the 5α reduction of testosterone there is apparently no substantial local activation at least on the basis of the evidence of cytosol receptor affinities in brain. Thus, the binding affinities for testosterone and DHT measured in brain cytosol by different observers are in the same order of magnitude [1–4]; the presence in cytosol of one or other of DHT or testosterone in excess in the unlabelled form effectively inhibits binding of the labelled species of the other substance [4] and, furthermore, nuclear uptake and retention of both DHT and testosterone has been demonstrated [5–7]. Even if effects of testosterone in castrated rats on mating behaviour or gonadotrophin secretion can be simulated by oestradiol or DHT either alone or in combination (and this does not seem to be true for all effects in all species) it does not follow that testosterone, *per se*, never is the active agent. This, clearly, could be the case if androgen responsive cells were to lack both aromatising and the reductase enzyme systems. Nevertheless, the possibility of the existence of binding specificity in brain cytosol that might sharply differentiate testosterone and DHT did not seem to have been rigorously excluded.

METHODS

In our experiments we have used cytosols prepared from hypothalamus, amygdaloid region, neocortex and ventral prostate of 2 and 3 day orchidectomised adult rats and from the same brain regions in intact adult females. Binding equilibrium at 0° was found in preliminary experiments to be reached somewhat faster with DHT than with testosterone and the incubation time of 4 h which was sufficient for equilibrium with testosterone, was used with both ligands. Binding was measured by separation of bound ligand on small columns of Sephadex LH20 [8]. Estimates of binding parameters were made in each cytosol prepared for both testosterone and DHT, either by constructing Scatchard plots of the difference in total binding and binding in the presence of 50 or 100-fold excess of unlabelled ligand or by applying the graphical curve fitting method of Rosenthal to the Scatchard plot of total binding [9–10]. The latter method had the advantage that parallel incubations were unnecessary and because we had the impression that, at the higher concentrations of cold testosterone, binding in low affinity complexes was tending to saturation.

RESULTS AND DISCUSSION

Figure 1 shows individual estimates of the abundance of the low capacity, high affinity binding sites for one ligand plotted against the estimates for the

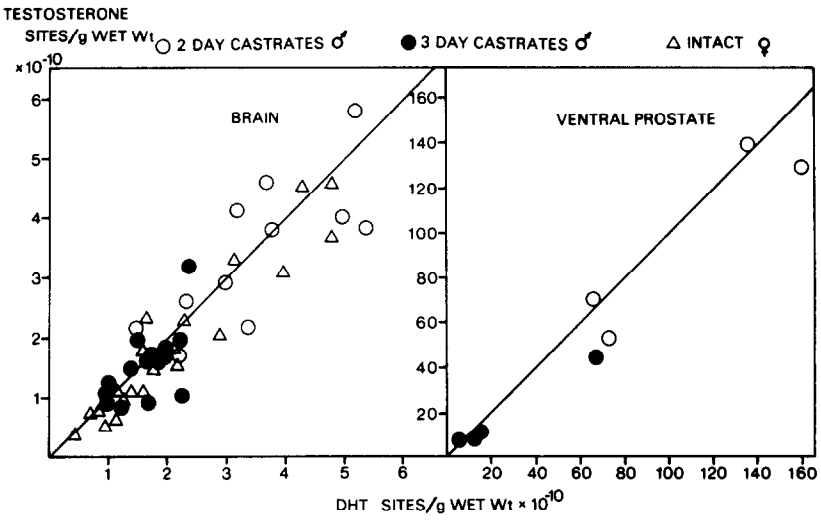

Fig. 1. Estimates of binding capacities for dihydrotestosterone and testosterone measured separately in the same cytosols from brain regions (amygdala, hypothalamus and cortex) in castrate male rats or intact females and in ventral prostate of castrate male rats.

other ligand in the same cytosol. The left hand frame shows the combined values from the three brain regions in the castrate males and females and the right hand frame gives the values from ventral prostate cytosol. The lines shown are not the calculated lines of best fit for the points but are merely drawn to pass through the origin with Slope = Unity. In fact, the points are well fitted by the lines showing that variation in the abundance of sites for one ligand is paralleled by variation in the abundance of the other and, within the limits of error of such estimates, the parallelism appears to be exact. Since binding sites are more numerous in the cytosols from the 2 day castrates than in the 3 day castrates, the parallelism can be seen over ranges of values in which the extremes differ by more than 10-fold in brain or ventral prostate of over a 300-fold range if all the values are consolidated.

Table 1 shows mean values for estimates of dissociation constant of the limited capacity binding for the two ligands, measured separately in each cytosol. Although the affinities are in the same order of magnitude that for testosterone is always the lower value and there is no evidence of any difference between the 2 day and 3 day castrates. An apparently consistent feature is that estimates of affinities for both ligands are lower in prostate cytosol than in brain cytosol: however, we would not, at this stage, suggest that this represents a real difference in receptor properties. Thus, taking the two aspects of these experiments, the measurement of site numbers and their affinities, there is no evidence for the independent occurrence of receptors with greater or lesser avidity for one or other of the two ligands.

The thermolability of androgen receptors is well known and great care was taken in all operations that temperature of the tissue, the homogenates and the cytosols did not exceed 2°. Figure 2 shows Scatchard plots for the low capacity high affinity binding of DHT in cytosols from hypothalamus and amygdala of 3 day castrate males exposed to preincubation for 1 h at 0°, 18° and 30°, before addition of ligand.

Table 1. Estimates of equilibrium dissociation constants of the high affinity binding of dihydrotestosterone and testosterone measured separately in the same cytosols from brain and ventral prostate of rats

		K_D(M × 10^{10})		
		Testosterone	DHT	T/DHT Paired mean
Male				
2 day castrates				
brain	(12)	4.74	2.26	2.20 ± 0.21
ventral prostate	(4)	6.23	4.60	1.36 ± 0.11
3 day castrate				
brain	(15)	4.20	2.24	1.89 ± 0.15
ventral prostate	(4)	6.35	4.59	1.59 ± 0.32
Female				
brain	(21)	3.51	2.41	1.53 ± 0.14

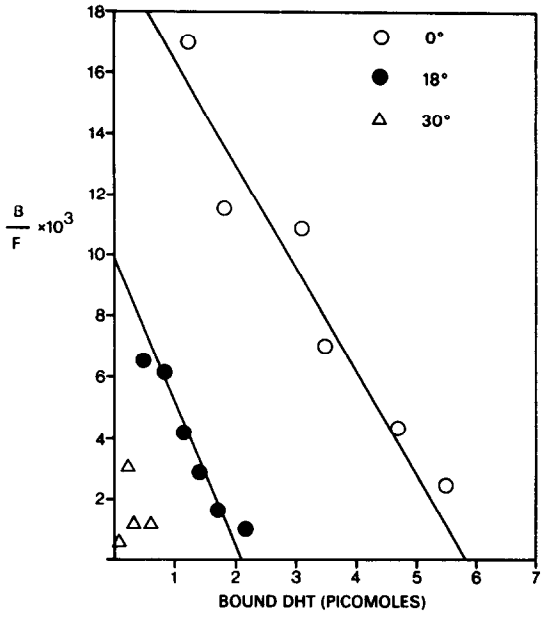

Fig. 2. Scatchard plots of high affinity binding of dihydrotestosterone in brain cytosols (hypothalamus and amygdala) from 3 day orchidectomised rats. Before incubation with ^{3}H labelled ligand for 2 h at 0° the cytosols were allowed to stand for 1 h at 0°, 18° or 30°.

All the binding activity has disappeared in the cytosol preincubated at 30° and that incubated at 18° has only about 1/3rd of the binding capacity of the cytosol kept at 0°, although the affinity of the remaining sites appears to be unaltered.

The results of an experiment in which the order of events was reversed are shown in Fig. 3. That is, the cytosols were incubated with labelled ligand to equilibrium at 0° for 4.25 h and then, either transferred to 26° or kept at 0° for a further 45 min. In brain cytosol, exposure of the DHT complex formed at 0° to the higher temperature seems not to have affected binding at all. In ventral prostate cytosol there seems to be a small degree of dissociation of the complexes but the saturation capacity is unchanged. Thus the DHT/receptor complex in both brain and ventral prostate appears to be stable in conditions that render free receptor inactive. The parallel experiments with testosterone using aliquots of the same cytosols, contrast sharply with those with DHT in that when the preformed testosterone complex is exposed to 26° no detectable binding remains. The point of this experiment is that it is possible to create conditions in which binding of testosterone appears to have been destroyed while

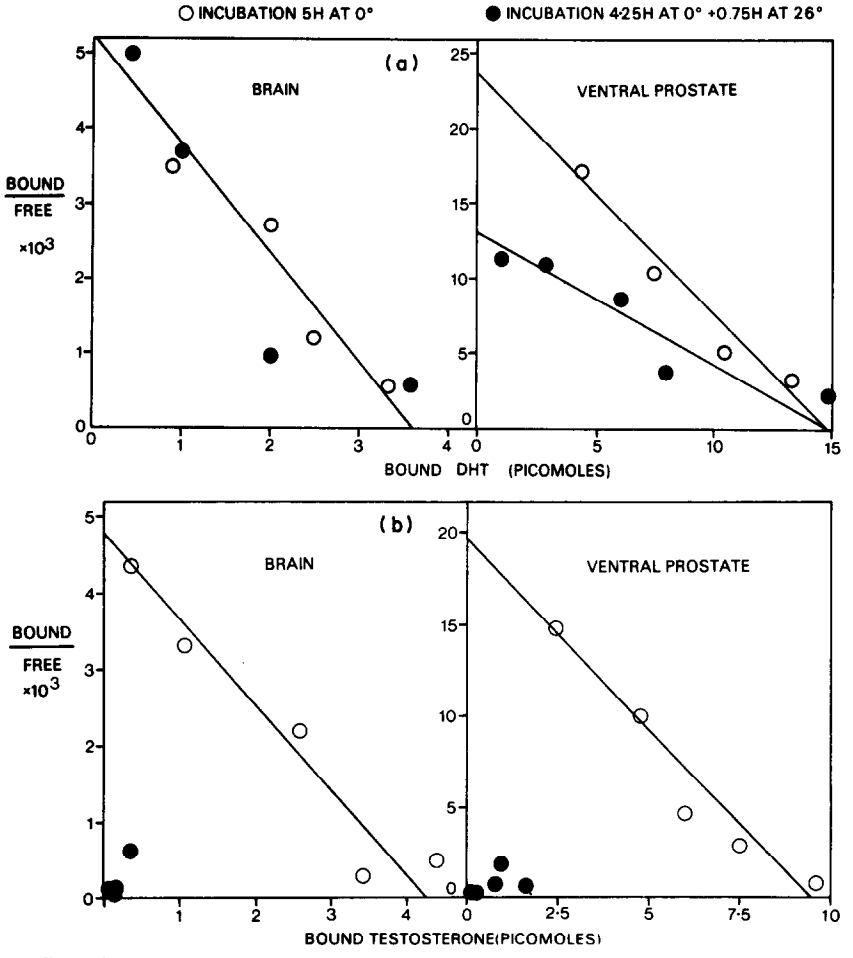

Fig. 3. Effect of exposure to 26° for 45 min on retention of (a) DHT or (b) testosterone in the bound state in previously formed complex with cytosol receptor.

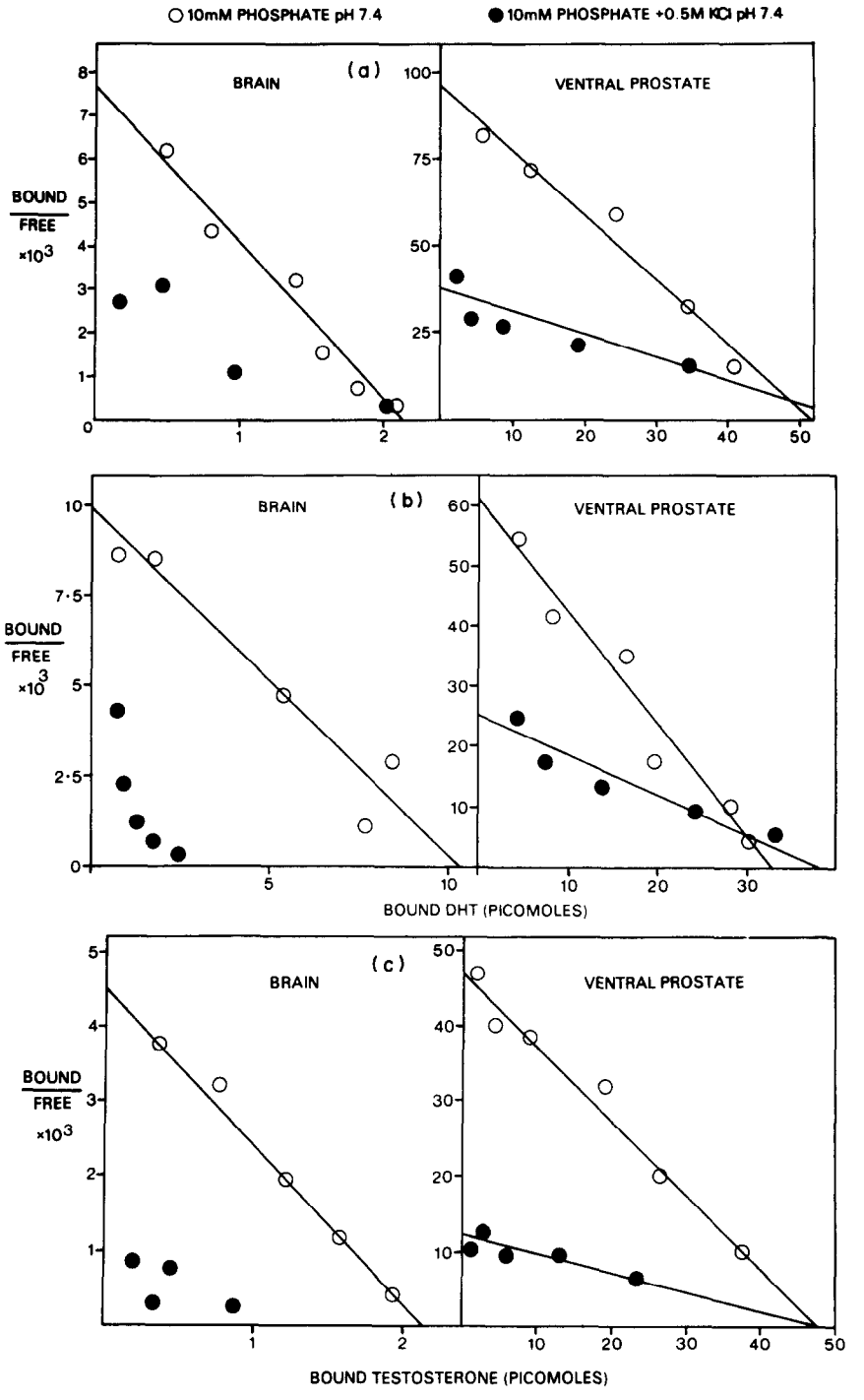

Fig. 4. Scatchard plots of high affinity binding of dihydrotestosterone and testosterone incubated at 0° in brain and ventral prostate cytosol with and without added KCl (0.5 M). (a) DHT: separation by LH20. (b) DHT: separation by hydroxylapatite. (c) testosterone: separation by LH20.

binding of DHT is not. But it would be mistaken to interpret this as demonstrating selective binding of DHT. It is more likely that the results arise from the combination of three factors—the stability of complexes formed with DHT and the lability at higher temperatures of both the testosterone complexes and of the free, uncomplexed binding sites.

Raising the temperature of DHT receptor complexes in extracts of ventral prostate cytosol to 20–30° is known to cause profound alterations in the complex. The S value is reduced and the iso-electric point is changed. Presumably similar changes can occur with the testosterone complexes, but, to my knowledge, that has not been investigated [11–12]. It is remarkable, however, that the temperature induced transformation should have so little effect on

the binding of DHT in the complex and so profoundly affect the binding of testosterone.

Following these results, we thought that it would be interesting to look at the binding of the two ligands in cytosol preparations with raised ionic strength, by addition of KCl to a concentration of 0.5 M. Figure 4(a) shows results obtained with DHT: KCl and ligand were added to cytosol simultaneously followed by incubation at 0° for 4 h. An effect can be seen of raised ionic strength on DHT binding in both brain and ventral prostate. The binding capacity at saturation is unchanged but the affinities are reduced by about 3-fold in both tissues. The change in binding of testosterone in the presence of KCl (Fig. 4(c)) follows the same pattern to that seen with DHT. There is no change in the saturation capacity and the decrease in affinity is even more pronounced, the reduction in the high ionic strength cytosols in both brain and ventral prostate being about 5-fold.

We were concerned lest these observations might be an expression of an artifact in the method used to separate bound from unbound steroid i.e. on Sephadex LH20 columns. We therefore repeated the experiment using another method—adsorption of the bound complex on hydroxyapatite (Fig. 4(b)). The results with ventral prostate are very similar to those obtained using Sephadex LH20. The binding of DHT in brain cytosol with KCl is so small that we are reluctant to attach any significance to the apparently high affinity of a small number of binding sites, that the results suggest.

Finally, to re-iterate our conclusion: the highest affinities for both ligands are obtained in conditions where the complexes are formed in the highest state of aggregation and although it is possible to create conditions in which affinity for testosterone is decreased to a greater extent than that for DHT, there is no evidence for separate receptors for the two steroids.

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