Characterization of [^{3}H]Dihydroergocryptine Binding Sites in Brown Adipose Tissue. Evidence for the Presence of α-Adrenergic Receptors *

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The main function of brown adipose tissue (BAT) is to generate heat during cold-stress in hibernating animals and in the newborn. The calorigenic response follows noradrenergic stimulation of adenylate cyclase, probably primarily a β-adrenergic receptor mediated activation, cAMP-production, lipolysis and an increase in mitochondrial oxygen consumption. The β-receptor of BAT has been characterized recently.[1,2] Nonetheless it has been suggested that an α-adrenergic receptor could also be involved in thermogenesis,[3-5] although playing probably only a minor role. In the light of this possibility of an α-receptor population in BAT, we decided to examine whether this was the case. We have used a very potent α-antagonist, [^{3}H]dihydroergocryptine (DHE), in order to monitor these sites by a direct binding approach.[6]

Results and discussion. Fig. 1 a shows the binding curve from one representative experiment made in duplicate. Half-maximal binding occurred at about 0.25 nM DHE and the binding sites were saturated at about 10 fmol/mg protein. For more accurate determination of the dissociation constant, K_D, and maximal binding, B_{max}, the data were analysed according to Scatchard.[7] The data (Fig. 1 b) fitted quite well to a straight line indicating one single set of binding sites. The K_D was 0.48 nM and B_{max} 10.6 fmol/mg protein. However, we cannot preclude that at higher concentrations of DHE (> 15 nM) another site of relatively lower affinity could be present in our homogenate preparation. This aspect will be investigated, because the presence of two subtypes of α-receptors has been reported.[8]

DHE associates very rapidly with its binding site

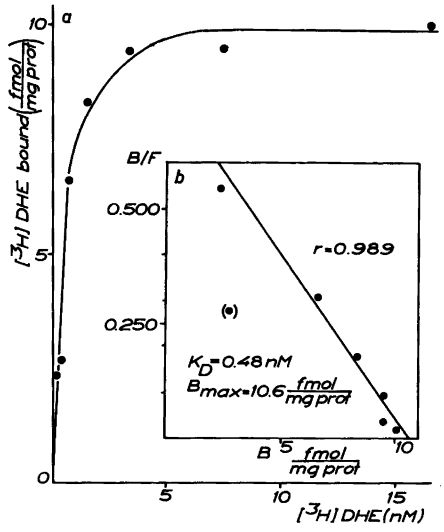

Fig. 1. [^{3}H]dihydroergocryptine (DHE) binding to brown adipose tissue membranes as a function of radioligand concentration. a. Specific binding as a function of the total DHE concentrations. Conditions as described in Experimental; b. Scatchard plot of specific DHE binding found in Fig. 1 a. *r* is the correlation coefficient.

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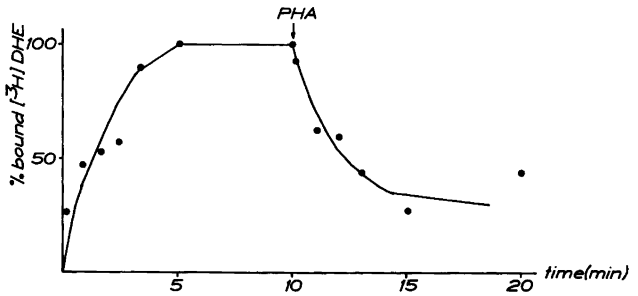

Fig. 2. Time course of specific DHE association to and dissociation from brown fat membranes. *PHA* = 43 μM phentolamine. Conditions as described in Experimental.

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($t_{1/2} \cong 1.5$ min, Fig. 2) and upon addition of a high excess of phentolamine it also dissociates rapidly ($t_{1/2} \cong 2.5$ min, Fig. 2).

Our laboratory has previously found that there are 48 fmol β-adrenergic receptors per mg protein as measured by the very potent β-antagonist, (−)[^{3}H]dihydroalprenolol (manuscript in preparation). This figure should be compared to the one reported here of 10 fmol bound DHE per mg of the same homogenate preparation. In the light of this difference, one may speculate about the relative contribution of α- and β-pathways in the calorigenic response in brown adipose tissue. This could possibly indicate that the α-pathway plays a minor role or mediates some other function. This remains challenging to investigate. In conclusion, brown adipose tissue appears to contain a population of binding sites which bind the highly potent α-antagonist,[^{3}H]dihydroergocryptine.

Experimental. A BAT homogenate was prepared from hamsters. Pieces of tissue were minced with scissors and homogenised in 0.25 M sucrose, 10 mM Tris−HCl, pH 7.4, 1 mM EDTA. The 10 % homogenate was centrifuged at 4 °C for 30 min at 100 000 *g*. The pellet was rehomogenised, washed three times and centrifuged at 4 °C for 30 min at 100 000 *g*. The final pellet was suspended in 50 mM Tris−HCl, pH 7.4, 10 mM $MgCl_2$ (incubation buffer, see below) to a protein concentration of about 10 mg/ml. This preparation was used for the binding assay. The binding assay was performed in principle as in Ref. 6 by incubating various concentrations of DHE for 15 min at 37 °C. The two agents used, DHE and phentolamine, were diluted to the proper concentration in a "dilution medium" (an aqueous solution containing 5 mM HCl and 9.6 % ethanol) from fresh stock solutions. The assay was performed in a total volume of 300 μl: 150 μl incubation buffer, 50 μl "dilution medium" or 50 μl 2.6×10^{-4} M phentolamine and 50 μl homogenate suspension. The incubation was started by addition of 50 μl of the proper concentration of DHE. The binding reaction was stopped by adding 3 ml ice-cold buffer and immediately filtered through a Whatman GF/C glass fiber filter. The filter was rinsed with 20 ml ice-cold buffer.

Non-specific binding was determined as the remaining binding in the presence of phentolamine (43 μM). Alternatively the level of non-specific binding was calculated according to eqn. (1),[9] where F

$$B_s = B_t - F(\lim_{B_t \to \infty} B_t/F) \quad (1)$$

is the free concentration of (^{3}H)DHE and B_s is the specific binding. This correction was done from total binding, B_t, analysed according to Scatchard.[7] The association rate was determined in homogenate incubated as above, containing 3.05 nM DHE, and taking 0.3 ml aliquots for rapid filtration. The dissociation rate was determined after the reaction mixture had come to equilibrium, and an excess of phentolamine had been added.

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