matters arising

Dihydroergocryptine is a non-selective antagonist for human platelet α-adrenoreceptors

BINDING of ^{3}H-dihydroergocryptine (DHEC) which is suppressible by phentolamine is widely used as a measure of the α-adrenoreceptor content of tissues[1]. Recently, Kunos *et al.*[2] have reported that phenoxybenzamine, an irreversible α_1-selective adrenoreceptor antagonist, suppressed only a portion of the specific DHEC binding to rabbit uterine membrane fractions although this antagonist had caused total blockade of noradrenaline-induced contraction in the rabbit uterine strips from which the membrane fractions had been prepared. These data suggest that not all the α-adrenoreceptors present in rabbit uterus may be involved in mediating contraction induced by noradrenaline but do not define the nature of the additional α-receptors detected[2].

Using appropriate selective α-adrenoreceptor agonists and antagonists we have recently demonstrated in a functional test system that human platelets carry both α_1- and α_2-adrenoreceptors[3]. In appropriate conditions these cells can therefore be used to analyse the selectivity of α-agonists and antagonists for which this property has not been defined. In the case of an antagonist, selectivity is most appropriately analysed by comparing the potency of the drug as an inhibitor of the clonidine (α_2-adrenoreceptor-mediated) and methoxamine (α_1-adrenoreceptor-mediated) stimulation of the response to ADP. When such studies are carried out using DHEC, the data obtained (Fig. 1) clearly demonstrate that this antagonist has very little ability to discriminate between the platelet α_1- and α_2-adrenoreceptors although it seems to be to be slightly more potent as an inhibitor of the α_1-mediated response. The data of Fig. 1 closely resemble those obtained in similar studies using the classical non-selective α-adrenoreceptor antagonist, phentolamine. Peroutka *et al.*[5] have reported that DHEC is also non-selective with respect to the proposed 'agonist' and 'antagonist' conformations of the α-adrenoreceptor in calf brain.

If the lack of α-selectivity of DHEC as defined here for human platelets is applicable to other tissues, as seems to be the case for uterine smooth muscle[2], caution should be exercised when this ligand is used to detect a correlation between a specific biological effect and receptor content. Thus, if the tissue contains significant concentrations of both α_1- and α_2-adrenoreceptors, estimation of receptor number from the extent of ^{3}H-DHEC binding will give misleading results unless selective α_1- and α_2-antagonists are used to indicate specificity. Similar considerations apply in studies which report changes in the number of α-adrenoreceptors present resulting, for example, from prior exposure of the cells to catecholamines[6].

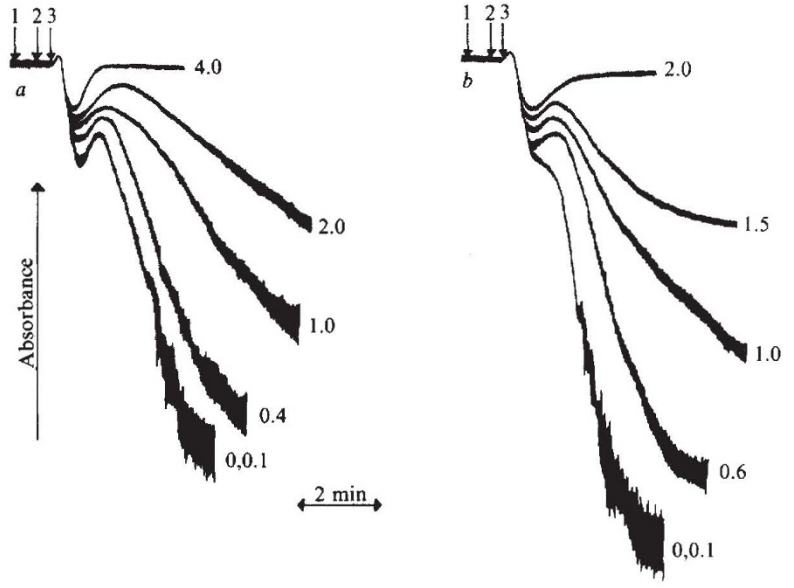

Fig. 1 Inhibition by DHEC of the stimulation of the response of human platelets to ADP induced by clonidine (*a*) or methoxamine (*b*). Aggregation was measured in 0.25-ml samples of human platelet-rich plasma prepared as described previously[4]. Additions were made as follows: at 1, DHEC at the concentrations (μM) indicated by the numbers on the trace; at 2, 5.7 μM clonidine (*a*) or 80 μM methoxamine (*b*); at 3, 1 μM ADP. Unpublished studies similar to those published previously[3] demonstrate that methoxamine stimulation of the response to ADP is mediated by an α_1-adrenoceptor. DHEC at concentrations up to 5 μM had no effect on the response to 1 μM ADP added alone.

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Are cardiac muscle cells skinned by EGTA or EDTA?

MILLER[1] re-examined criteria that have been used in defining the state of frog cardiac membranes treated with EGTA or EDTA. From these data, he concluded that 'supposedly' skinned cardiac cells are not skinned by EGTA/EDTA treatments.

To assess whether a preparation is skinned or not, it is helpful to have an objective definition of a skinned fibre preparation. This is especially necessary when using chemical methods of preparation that do not, as in mechanically skinned preparations, remove the major portion of sarcolemmal membranes. A definition we have found useful is that a fibre is skinned when normally impermeable solutes such as MgATP, EGTA, EDTA and other large molecular weight ions gain free access to the myofilament space.

Under this definition, critical physiological tests to determine whether a fibre is skinned must include one or more of these normally impermeable solutes, and a means for evaluating whether the solute has gained free access to the myofilament space. Examples that meet requirements of a 'critical test' for whether a preparation is skinned are (1) development of rigor and relaxation from rigor should occur within seconds of MgATP removal or addition, respectively (time interval predicted for ATP diffusion into or out of the fibre core)[2]; (2) the response of chemically skinned fibres to variations in low MgATP concentrations (0.1–50 μM) should be identical to that of mechanically skinned fibres and consistent with data[3] obtained on extracted myofibrillar preparations; (3) the responses of chemically skinned fibres measured in (1) or (2) should not be quantitatively affected either by mechanical removal of the sarcolemma or by treatment of the pre-

0028-0836/79/340700—03$01.00