Monoclonal antibodies that inhibit somatomedin-like activity

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The somatomedins are insulin-like growth factors that are thought to mediate the actions of pituitary somatotropin (growth hormone) on cartilage and possibly other tissues (Phillips & Vassilopoulou-Sellin, 1980a,b). Pure preparations of somatomedins are not widely available, which limits the production of highly specific antisera to these factors. Monoclonal antibodies to somatomedin C has been described by Baxter (1982), who used ability to bind $^{125}$I-labelled somatomedin C in screening for antibody-producing hybridomas. The ability of this antibody to block the biological actions of somatomedins was not reported.

An alternative approach to the preparation of monoclonal antibodies to somatomedin C has been described by Baxter et al. (1982), who used ability to bind $^{125}$I-labelled somatomedin C in screening for antibody-producing hybridomas. The ability of this antibody to block the biological actions of somatomedins was not reported.

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**Fig. 1. Inhibition of bioassayable somatomedin-like activity in human serum by media conditioned by hybridomas F7A and F7B**

Cartilage discs were incubated with 30% (v/v) human serum and medium RPMI (unconditioned or conditioned by hybridoma cells) at the concentration shown. Incorporation of $^{35}$SO$_4^{2-}$ into six discs (mean ± S.E.M.) is shown.
The specificity of binding of somatotropin (growth hormone) and prolactin to purified plasma membranes from rabbit liver

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Specific binding sites for lactogenic and somatogenic hormones are found in various tissues of vertebrates. The most important target for lactogenic hormones in mammals is the mammary gland, but the prime target tissue of somatogenic hormones is less well defined. However, many of the anabolic effects of somatotropin (growth hormone) are mediated by the somatomedins, a major production site of which is the liver, suggesting that the liver represents a major target for somatotropin action. Posner et al. (1974) and Kelly et al. (1974) have demonstrated the wide distribution of receptor sites for lactogenic and somatogenic hormones; membranes prepared from the liver of late-pregnant rabbits are a particularly rich source of somatogenic receptors.

There has been some disagreement about the nature of the somatotropin receptors in the liver. Posner et al. (1974), Herington et al. (1976) and Bonifacino et al. (1981) demonstrated a predominance of lactogenic receptors in rat liver. Tsushima & Friesen (1973) originally reported that binding sites for human somatotropin in rabbit liver are somatogenic, but later work indicated that lactogenic receptors (to which human somatotropin, but not non-primate somatotropins, can bind) also occur in rabbit liver (Posner et al., 1974; Parke & Forsyth, 1975; Waters & Friesen, 1979; Fix et al., 1981; Haupertle et al., 1983). Our own previous work (Cadman & Wallis, 1981) and that of Hughes (1979) suggests that the situation in membrane-bound receptor preparations from the liver of the pregnant rabbit is more complex. Various species were very similar, suggesting that solubilization does not substantially affect the binding properties of the receptors. Bovine and sheep somatotropins displaced 125I-labelled human somatotropin with almost the same potency as unlabelled human somatotropin. Various other non-primate mammalian somatotropins (including the rabbit hormone) competed for receptors with much lower potency. Bovine prolactin was more potent than sheep prolactin, but both were fairly effective in displacing 125I-labelled human somatotropin from receptors, whereas several other prolactins showed very low potency in this system.

Discussion

Previous work has shown that the binding of somatotropins and prolactins from various species to liver-membrane receptors from the late-pregnant rabbit does not accord closely with either the growth-promoting or the lactogenic properties of the hormones (Hughes, 1979; Cadman & Wallis, 1980; Fix et al., 1981; Wallis & Cadman, 1981; Haupertle et al., 1983). Such studies suggest that the liver contains several different receptors for hormones of the somatotropin/prolactin family, only some of which mediate growth-promoting or lactogenic actions. This could have been partly explained by the heterogeneity of the microsomal membrane preparation used for the binding studies. However, the results obtained here, with receptors from purified plasma membranes, are very similar to those obtained previously with microsomal membranes (Cadman & Wallis, 1981). We conclude that the heterogeneity of the membrane preparation was not contributing to the complex results obtained previously, and that this complexity reflects the nature of the plasma-membrane-associated receptors themselves.

It has been suggested that the predominant binding sites for human somatotropin are not involved in mediating the lactogenic or somatogenic properties of prolactin or somatotropin in the rabbit. The fact that rabbit somatotropin and prolactin are poor displacers of 125I-labelled human somatotropin/prepared by the 'iodogen' method of Fraker & Spec (1978), as described by Cadman & Wallis (1981). Plasma membranes from the liver of pregnant rabbits were isolated by the method of Neville (1968), with scaling up as described by Goldstein & Blecher (1976) to increase the yield. Samples were taken at each stage of purification to test for the purity of plasma membranes by means of marker-enzyme assays and for binding studies. Plasma membranes were solubilized with 0.5% (v/v) Triton X-100 (Shiu & Friesen, 1974; Herington & Veith, 1977; Cadman & Wallis, 1981). The binding of 125I-labelled human somatotropin to purified plasma membranes was time- and temperature-dependent. Binding was slower at 4°C than at 25°C. Binding of human somatotropin to purified plasma membranes was increased by bivalent cations, Ca2+ being more effective than Mg2+. In the presence of the univalent cation Na+, binding was no greater than in the absence of metallic cations. Use of the non-ionic detergent Tween 20 (polyoxyethylene sorbitan monolaurate) in the binding assay instead of bovine serum albumin increased the apparent specific binding by lowering the non-specific binding by half. Increasing the concentration of plasma membranes increased specific binding to a maximum of 52% of the total radioactivity added.

Curves showing displacement of 125I-labelled human somatotropin from purified plasma membranes and solubilized plasma-membrane receptors by somatotropins and prolactins from various species were very similar, suggesting that solubilization does not substantially affect the binding properties of the receptors. Bovine and sheep somatotropins displaced 125I-labelled human somatotropin with almost the same potency as unlabelled human somatotropin. Various other non-primate mammalian somatotropins (including the rabbit hormone) competed for receptors with much lower potency. Bovine prolactin was more potent than sheep prolactin, but both were fairly effective in displacing 125I-labelled human somatotropin from receptors, whereas several other prolactins showed very low potency in this system.

Methods and Results

125I-labelled human somatotropin was prepared by the 'iodogen' method of Fraker & Speck (1978), as described by Cadman & Wallis (1981). Plasma membranes from the liver of pregnant rabbits were isolated by the method of Neville (1968), with scaling up as described by Goldstein & Blecher (1976) to increase the yield. Samples were taken at each stage of purification to test for the purity of plasma membranes by means of marker-enzyme assays and for binding studies. Plasma membranes were solubilized with 0.5% (v/v) Triton X-100 (Shiu & Friesen, 1974; Herington & Veith, 1977; Cadman & Wallis, 1981).