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Recombinant γ-interferon stimulates iodide uptake and cyclic AMP production by the $FTRL_5$ thyroid cell line

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The effect of recombinant rat γ-interferon (γ-IFN) on iodide uptake and cAMP production by rat thyroid cells in vitro was studied using the continuously growing, functional $FRTL_5$ cell line. Both functions were stimulated by γ-IFN at concentrations of 1–10 U/ml. Iodide uptake was dependent on protein synthesis, since it was blocked by cycloheximide treatment, but was not dependent on growth factors in calf serum routinely used for $FRTL_5$ cell culture. These results show that γ-IFN can stimulate thyroid cell function as well as aberrant Ia expression in vitro.

Thyroid; Iodide uptake; cyclic AMP production; γ-Interferon; (Rat)

1. INTRODUCTION

The continuously growing, differentiated rat thyroid cell line, $FRTL_5$, has been used extensively to investigate thyroid cell function in vitro, in particular cyclic AMP (cAMP) production and iodide uptake in response to recognised stimulators such as thyrotrophin (TSH) and TSH receptor antibodies [1-4]. Earlier work has established that mouse interferons stimulate both adenylate cyclase activity in rat thyroid membranes [5] and iodide uptake by $FRTL_5$ cells [6]. However, these experiments used type I β-IFN which differs in many respects from type II γ-IFN.

γ-IFN is produced by activated T cells and has a wide range of immunological effects [7]. It is known that γ-IFN is produced by activated T cells infiltrating the thyroid in various types of autoimmune thyroiditis [8] and seems to be the key modulator responsible for inducing aberrant Ia antigen expression on the thyroid in these conditions, whereas α- and β-IFN do not have this property [9]. We have recently found that recombinant rat γ-IFN will induce Ia antigens on $FRTL_5$ cells grown in vitro (Weetman et al., submitted) and have now investigated whether this type of IFN will modulate $FRTL_5$ iodide uptake and cAMP production.

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2. MATERIALS AND METHODS

The $FRTL_5$ cells were obtained from Dr N. Marshall (University College Hospital, London) and used within 20 passages. They were maintained as described in [1,2] in Coon's modified Hams F-12 medium with 5% newborn calf serum and six hormone (6H) supplements: TSH (10 mU/ml), insulin (10 μg/ml), cortisol (10^{-8} M), transferrin (5 μg/ml), glycyl-L-histidyl-L-lysine (10 μg/ml) and somatostatin (10 μg/ml). 5H medium lacked TSH.

Assays were performed in replicates of six in 24-well plates (Nunc, Kamstrup, Denmark), after resting the cells for 7 days in 5H medium. In assays for iodine uptake, stimulators were added for 48 h in fresh 5H medium and iodine uptake was measured 1 h after the addition of 0.1 μCi carrier-

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free $Na^{125}I$, using the perchlorate discharge method in [10]. The production of cAMP was measured over a 4 h period after addition of the stimulator in low-salt medium [11]; cAMP in the supernatant was determined using an in-house radioimmunoassay, with ^{125}I-cAMP kindly provided by Dr N. Marshall. In all cases the residual cell mass was determined by crystal violet staining of the plate [12]; variation between wells was never greater than 7%, so no correction was made for cell number. Recombinant rat γ-IFN [13] was obtained from Holland Biotechnology and recombinant human γ-IFN from Genentech. Statistical evaluation was by Student's t-test.

3. RESULTS

3.1. *Iodide uptake*

Iodide uptake was significantly enhanced by rat γ-IFN at concentrations as low as 0.1 μg/ml; human γ-IFN had no effect (fig.1). Moreover, γ-IFN had an additive effect on the iodide uptake stimulated by TSH (fig.2). The action of γ-IFN on iodide uptake was sensitive to cycloheximide. Pretreatment of the cells with 50 μM cycloheximide for 1 h before the addition of 10 U γ-IFN/ml for 48 h reduced iodide uptake, expressed as a percentage increase over basal values, from 566 ± 30 to 372 ± 22% ($P<0.001$). However, pretreatment with phorbol myristate acetate (10 or 100 ng/ml) had no effect on iodide accumulation (values for γ-IFN stimulated iodide uptake within 20% of those for cultures with γ-IFN and phorbol ester: $P>0.05$). To examine the duration of γ-IFN exposure required for an effect on iodide uptake, 7 day cultures in 5H medium were treated with γ-IFN for varying periods, washed 5 times and then culture continued in 5H medium for a total time of 44 h. The addition of γ-IFN (10 U/ml) for periods as short as 1 h prior to transfer to 5H medium alone produced a significant effect, with iodide uptake 19 ± 10% (mean ± SD) of the maximal value produced by continuous culture for 44 h in γ-IFN. Exposure for 4 or 9 h gave respective iodide uptake values of 48 ± 14 and 89 ± 15% of the maximum.

The ability of γ-IFN to stimulate iodide uptake in serum-free medium was tested, in view of the possibility that constituents of the serum such as growth factors could be contributing to the results obtained. Cells were incubated in serum-free 5H

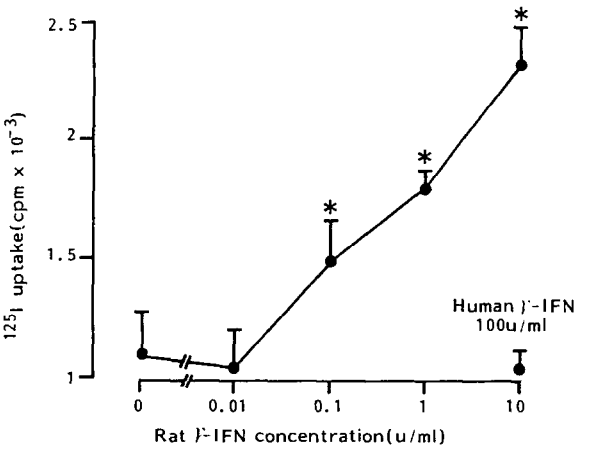

Fig.1. Effect of γ-IFN concentration on ^{125}I uptake by $FRTL_5$ cells. Bar represents 1 SD. * $P<0.001$ vs cultures without γ-IFN.

medium with γ-IFN at 10 U/ml and although a reduction in iodide uptake was observed compared to samples treated with γ-IFN in 5H medium with 5% calf serum (mean ± SD increase in uptake over basal 401 ± 13 vs 558 ± 47%; $P<0.001$) the cells were nevertheless stimulated to take up iodide significantly ($P<0.001$ vs basal control).

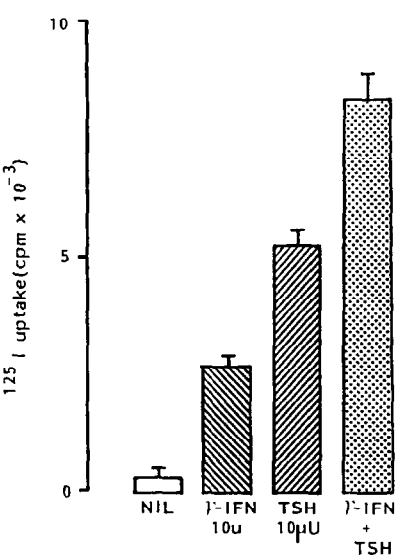

Fig.2. Effect of γ-IFN on TSH-stimulated iodide uptake by $FRTL_5$ cells. Bar represents 1 SD. The combination of γ-IFN plus TSH produced significantly greater stimulation than TSH alone ($P<0.001$).

Table 1

cAMP accumulation in $FRTL_5$ supernatant 4 h after addition of γ-IFN or TSH

Addition	cAMP production (pmol/culture)
Nil	5 ± 1
Rat γ-IFN (10 U/ml)	32 ± 3[a]
(1 U/ml)	26 ± 3[a]
Human γ-IFN (100 U/ml)	6 ± 1
TSH (50 μU/ml)	81 ± 5[a]
(5 μU/ml)	62 ± 5[a]
TSH (50 μU/ml) + γ-IFN (10 U/ml)	116 ± 8[a]
TSH (5 μU/ml) + γ-IFN (1 U/ml)	98 ± 6[a]

[a] $P < 0.001$ vs control

Results are means ± SD of six replicates

3.2. *cAMP production*

The accumulation of cAMP in $FRTL_5$ supernatants is detailed in table 1. Both rat γ-IFN and TSH stimulated cAMP production; there was an additive response when these agents were added together.

4. DISCUSSION

These results show that recombinant homologous γ-IFN stimulates iodide uptake and cAMP production by the rat thyroid cell line $FRTL_5$. It is known that TSH mediates $FRTL_5$ iodide incorporation by the cAMP second messenger system and that iodide uptake is also dependent on protein synthesis (inhibited by cycloheximide) but, unlike α_1-adrenergically regulated functions in $FRTL_5$ cells, is not affected by phorbol myristate acetate, which mimics the effect of diacylglycerol on protein kinase C [14,15]. The effect of γ-IFN on $FRTL_5$ cells was thus similar to TSH in these respects, although the kinetics of the response are somewhat slower since 64% of maximal iodide uptake is produced by TSH after exposure for only 1 h, and maximal uptake is found after 5 h [4].

Recently, another cytokine of immunological importance, interleukin-1, was shown to stimulate $FRTL_5$ DNA synthesis, although this activity was greatly enhanced by insulin-like growth factor-1, a known constituent of the calf serum routinely used for $FRTL_5$ culture [16]. γ-IFN was able to stimulate iodide uptake in the absence of calf serum and it is not clear at present whether the modest increase in stimulation seen in the presence of calf serum reflects a similar type of interaction or a non-specific effect of culture in a more optimal medium.

It has previously been found that purified (but not recombinant) type I IFN, apparently β-IFN, stimulated iodide uptake and cAMP production by $FRTL_5$ cells [6]. There is no structural homology between β-IFN and γ-IFN (type II IFN) and their effects are mediated by separate receptors; although the two types of IFN share certain activities, other effects are produced by a single IFN species [7]. Thyroid cells clearly possess both types of IFN receptor in view of the present results and the action of γ-IFN on thyroid cell Ia antigen expression [9]. However, only γ-IFN, and not α- or β-IFN, is able to mediate this latter activity ([9] and unpublished). It has been shown that the thyroid lymphocytic infiltrate in autoimmune thyroiditis (Graves' disease or Hashimoto's thyroiditis) is capable of producing γ-IFN, responsible for the aberrant thyroid Ia antigen expression seen in these conditions [8,9]. The present results suggest that local γ-IFN production by T cells could also have effects on thyroid function in these conditions.

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