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THE EFFECT OF FATTY ACID ON THE UPTAKE OF THYROXINE BY THE PERFUSED RAT HEART

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SUMMARY

1. A study has been made of the effect of fatty acids on the uptake of thyroxine by the perfused rat heart.

2. Oleic acid increases the thyroxine uptake of hearts perfused by solutions containing serum. In the absence of serum, oleic acid is without action.

3. The thyroxine uptake of hearts perfused by 0·25 % serum is approximately doubled by the addition of oleic acid at a concentration of 0·0025 m-equiv/l. An effect on thyroxine uptake, however, is demonstrable at only one twentieth of this concentration.

4. Long-chain fatty acids are much more effective than short-chain fatty acids.

INTRODUCTION

It has been proposed that the uptake of thyroxine by tissues is determined in part by the degree of which the hormone is bound on to specific binding proteins in the plasma (Ingbar & Freinkel, 1960; Robbins & Rall, 1960). Experimental evidence in favour of this view has been provided by studies using the perfused rat heart (Hillier, 1968*d*).

Tabachnick (1964) has shown that long-chain fatty acids inhibit the binding of thyroxine by human serum albumin and, in man, a correlation has been found between the free-thyroxine level and the concentration of fatty acid in the plasma (Hollander, Scott, Burgess, Rabinowitz, Merimee & Oppenheimer, 1967). The theory has therefore been proposed that the uptake of thyroxine by the tissues is determined by the extent to which the hormone is bound on to proteins in the blood and that this degree of binding is regulated by the concentration of fatty acid in the plasma (Hollander *et al.* 1967).

In this study an attempt has been made to test this theory, using the isolated, perfused rat heart.

METHODS

The methods used in this investigation have been described in detail in a previous paper (Hillier, 1968*c*). A further point, however, requires description.

Solutions of fatty acids were made as follows. The fatty acid (Hopkin & Williams and British Drug Houses) was dissolved in about 5 vols. of absolute ethanol and neutralized by an aqueous solution of sodium hydroxide (10 g/100 ml.) using bromothymol blue as indicator. The alcohol was evaporated off at 70° C and the solution made up to final volume with water. The stock solution of sodium oleate contained 0·2 m-equiv/l.

RESULTS

The effect of oleic acid on the uptake of thyroxine in the presence of serum. In the absence of thyroxine-binding protein in the perfusion fluid, the rat heart, after 30 min perfusion, accumulates radioactive thyroxine equivalent to 15 ml. fluid/g of heart. With 0·25 % serum in the perfusion fluid the total amount of thyroxine taken up is reduced to about 25 % of this value. In an experiment the effect of oleic acid on thyroxine uptake was examined in the perfused rat heart.

Five rat hearts were perfused by solutions containing radioactive thyroxine at a concentration of 1×10^{-4} μg/ml. and 0·25 % rat serum. After 30 min perfusion, when the system had come to equilibrium, the hearts were changed over to an identical solution containing in addition oleic acid (sodium salt) at a concentration of 0·025 m-equiv/l. Since the serum in the perfusion fluid was diluted 1:400 this concentration of oleic acid in whole serum would be 10 m-equiv/l. This is very considerably higher than the normal resting free fatty acid level in plasma, which is about 0·5 m-equiv/l. (Mallov, 1963).

The results are illustrated in Fig. 1. The hearts reached an initial equilibrium after 30 min perfusion by the first solution. When the perfusion fluid was changed to that containing oleic acid there was a further increase in uptake having a similar size and time course to the first increase.

When these experiments were repeated using perfusion solutions which did not contain any thyroxine-binding protein, the oleic acid was without effect on thyroxine uptake. This demonstrated that the fatty acid was very probably acting on the serum solution and not on the heart itself.

In another experiment a dose–response curve for this effect was established. The experiments were performed in an exactly similar way to that already described. The increase in thyroxine uptake after 30 min in the presence of oleic acid was expressed as a percentage of the uptake in the absence of the acid. In all cases the perfusion solutions were made up to contain 0·25 % rat serum. The concentration of oleic acid used was varied between 0 and 0·025 m-equiv/l. (equivalent to between 0 and 10 m-equiv/l. in whole serum).

The results are illustrated in Fig. 2. An effect of oleic acid on thyroxine uptake was demonstrable at a concentration of 0·00125 m-equiv/l. (equivalent to 0·5 m-equiv/l. in whole serum). The concentration of fatty acid already present in the serum at collection was not estimated but was probably about the same as this value (0·5 m-equiv/l. whole serum;

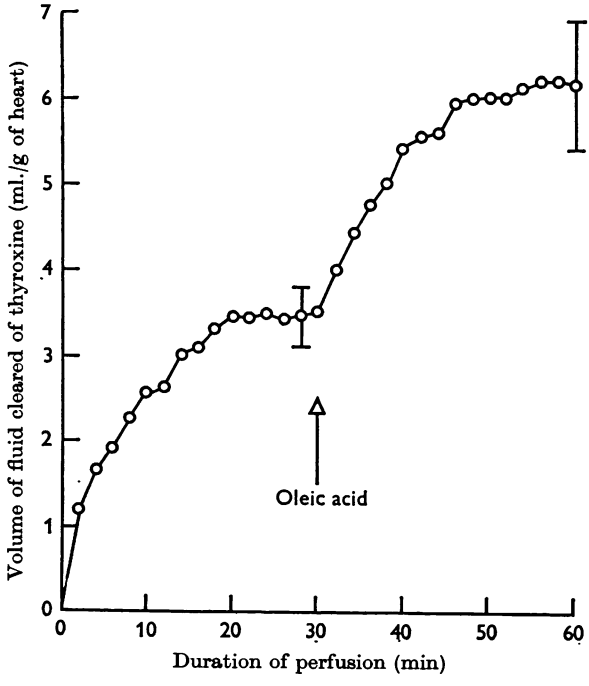

Fig. 1. The uptake of radioactive thyroxine by rat hearts. The perfusion fluid contained thyroxine, 1×10^{-4} μg/ml., and 0·25% rat serum. At the point indicated by the arrow the perfusion fluid was changed to one containing oleic acid in addition at a concentration of 0·025 m-equiv/l. The curve represents a mean of results from five separate hearts and the vertical lines indicate one standard deviation either side of the mean.

Mallov, 1963). The concentrations of oleic acid given in Fig. 2, refer only to added oleic acid and do not include this small amount of fatty acid already present. Provided, therefore, that the dilution factor is taken into account the above result illustrates that oleic acid is capable of influencing thyroxine uptake at concentrations which fall within the physiological range.

The effect of oleic acid on thyroxine uptake at different dilutions of serum. In the above experiment high dilutions of serum were used: first, because of the enormous amounts of serum that would otherwise be required;

secondly, with the simple experimental method employed, the hormone uptake occurring at high serum concentrations would hardly be measurable. In most experiments serum diluted 1:400 was used and it was assumed that the effect of oleic acid at a concentration of 0·02 m-equiv/l. in 0·25 % serum would be the same as that at a concentration of 10 m-equiv/l.

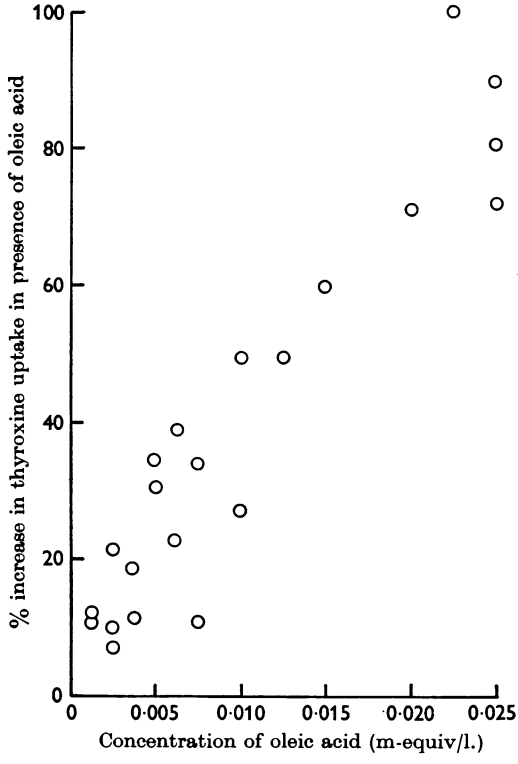

Fig. 2. A dose–response curve for the effect illustrated in Fig. 1. The percentage increase in thyroxine uptake 30 min after addition of oleic acid is plotted against the concentration of oleic acid used. The perfusion fluid in all experiments contained radioactive thyroxine 1×10^{-4} μg/ml. and 0·25 % rat serum. Each point represents an individual experiment on one heart.

in 100 % serum. This assumption was tested by experiments in which rat hearts were perfused by solutions containing different concentrations of serum and the percentage increase in thyroxine uptake 30 min after addition of oleic acid (0·0025 m-equiv/l.) was measured. The results are illustrated in Fig. 3. Each point represents one individual observation. It is clear that the magnitude of the response to oleic acid is dependent upon the concentration of serum used. At 0·25 % serum there is an 80 % increase in thyroxine uptake. This falls to about half at double the serum concen-

tration and about a quarter in 1 % serum. The shape of the curve suggests that the size of the response to oleic acid depends on the relative proportion of fatty acid to serum and that provided this proportion is maintained the response is independent of the dilution used. This latter point was examined further.

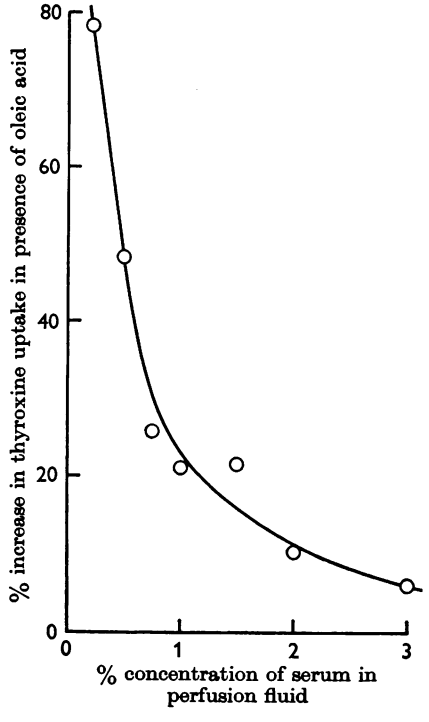

Fig. 3

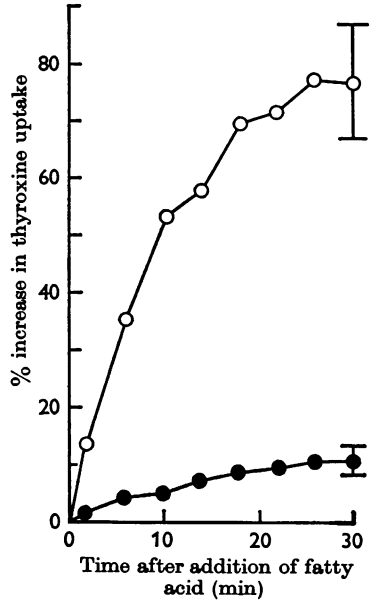

Fig. 4

Fig. 3. The percentage increase in thyroxine uptake by rat hearts 30 min after the addition of oleic acid to the perfusion fluid (0·025 m-equiv/l.). The dilution of serum was varied between 0·1 and 3 %. Each point represents an individual experiment on one heart. The line was drawn by eye. The concentration of thyroxine in the perfusion fluid was 1×10^{-4} μg/ml.

Fig. 4. The percentage increase in thyroxine uptake after addition of fatty acid to the perfusion fluid (0·25 m-equiv/l.). Each curve represents the mean of three hearts and the vertical lines are one standard deviation either side of the the mean. In all experiments the perfusion fluid contained 0·25 % rat serum. Oleic acid ○; butyric acid ●. The concentration of thyroxine in the perfusion fluid was 1×10^{-3} μg/ml.

The effect of oleic acid on thyroxine uptake was measured at different dilutions of serum. The concentration of oleic acid, however, was changed in proportion so that the value obtained was always equivalent to 10 m-

equiv/l. in whole serum. Under these conditions it was found that the proportionate increase in thyroxine uptake caused by oleic acid was independent of the serum concentration (the range of concentrations used varied between 0·1 and 10%). It was concluded that experiments done at high dilutions of serum could give results similar to ones using 100% serum provided that the concentrations of fatty acid were altered proportionately.

Comparison of long-chain and short-chain fatty acids. Tabachnick (1964) found that the physiologically occurring, long-chain fatty acids were most effective in inhibiting thyroxine binding by human serum albumin. An experiment was performed to determine whether the effect of fatty acid on thyroxine uptake also showed this specificity.

Rat hearts were perfused for 30 min by solutions containing radioactive thyroxine (1×10^{-3} μg/ml.) in 0·25% serum. The effect of fatty acid (0·025 m-equiv/l.) was then determined and the increase in uptake expressed as a percentage of the initial control value. The two acids studied were oleic acid (18 C atoms) and butyric acid (4 C atoms).

The results are illustrated in Fig. 4. Oleic acid was much more potent than butyric acid in increasing thyroxine uptake by the heart.

DISCUSSION

The ability of oleic acid to enhance thyroxine uptake by the perfused rat heart is very probably due to an inhibitory action on thyroxine binding by the serum proteins: first, oleic acid is without action in the absence of serum; secondly, direct measurements *in vitro* have shown that fatty acids (including oleic acid) can inhibit thyroxine binding by serum proteins (Tabachnick, 1964; Hollander *et al.* 1967). As yet, however, these results have been confined to work on human serum and plasma.

The concentration of free fatty acid in rat plasma is about 0·5 m-equiv/l. (Mallov, 1963). Addition of oleic acid sufficient to double this value causes a detectable increase in thyroxine uptake by the heart. It is possible, therefore, that the phenomenon is of some physiological significance, especially under those conditions where the plasma concentration of free fatty acids is raised.

It has been demonstrated that during cold exposure in the rat the biliary clearance of thyroxine is raised (Cottle, 1964; Hillier, 1968*a*). It has also been shown that the rate of thyroxine deiodination is enhanced during cold exposure, probably as a result of increased sympathetic activity (Hillier, 1968*b*). Upon exposure to cold the concentration of free fatty acid more than doubles (Mallov, 1963). This is a result of increased sympathetic activity (Himms-Hagen, 1967; Masoro, 1966). It is therefore possible that

the changes observed in thyroxine metabolism during cold exposure are induced by a reduction in thyroxine binding in the plasma caused by increased amounts of free fatty acid in the blood. The reduction in plasma thyroxine binding would accelerate the transfer of the hormone from the blood to the tissues, thereby enhancing its deiodination and biliary excretion. It is interesting to note in this context that serum from cold-adapted rats has a lower tri-iodothyronine-binding capacity than serum taken from warm-adapted rats (Cottle & Veress, 1966).

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