PAPERS ON CURRENT EXPERIMENTAL WORK

Metabolic and tissue effects of prolonged catecholamine infusion

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Summary

In recent years it has become clear that the free fatty acids of the plasma represent the major form of transport for lipid mobilized from the stored triglyceride in peripheral adipose tissue.

There is evidence that lipid mobilization is increased after trauma, burns and shock with depletion of the peripheral lipid stores and an increase in the circulating free fatty acids.

In this study lipid mobilization was induced in unanaesthetized dogs by continuous noradrenaline infusion at a rate of 1 μg/kg/min. A marked rise of the free fatty acids occurred and was associated with triglyceride deposition in tissues not normally the site of lipid storage. This change affected principally the liver, but to a lesser degree other organs including the lungs.

The possible significance of this lipid mobilization in the human setting is discussed.

Introduction

Catecholamine secretion is increased in patients with severe surgical disease, particularly when there is extensive soft tissue trauma or invasive sepsis. The contribution of the catecholamines in the cardiovascular response to injury is well known, but they have in addition effects on carbohydrate and lipid metabolism. Lipokinesis is enhanced, and the levels of free fatty acids (FFA) in the plasma rise. Dole (1956) and Gordon & Cherkes (1956) were the first to demonstrate that the FFA of the plasma are the major form of transport of fat from peripheral adipose tissue. Triglyceride in the peripheral depots is continuously being hydrolysed by the intracellular lipases of adipose tissue, and the FFA that are produced enter the circulation and are bound to albumin. They form only 2–3% of the total plasma lipid, their ordinary level being 500–600 μEq/l, but their turnover rate is very rapid at approximately 25% per minute (Havel & Fredrickson, 1956; Laurell, 1957; Dole & Rizack, 1961). In the postabsorptive state FFA are the principal substrate for all tissues except the red cells, the central nervous system and the adrenal medulla (Gordon, Cherkes & Gates, 1957; Bragdon & Gordon, 1958; Carlson & Ekelund, 1963). Circulating FFA enter cells by active transport and are either esterified and stored, or terminally oxidized for energy (Havel, 1962). The same two possibilities exist in the liver but here two additional pathways are available. Triglycerides synthesized in the liver may enter the circulation where they are carried in the low density lipoprotein fraction; this appears to be the major pathway for the transport of fat from the liver to other tissue sites (Havel, 1961). Ketones, formed by hepatic fatty acid oxidation, are returned to the blood stream, for oxidation in muscle and other tissues (Langdon, 1960).

The rate at which lipid is mobilized and FFA are formed is controlled by a number of interacting neural, hormonal and nutritional mechanisms. Lipid mobilization is stimulated by increased sympathetic activity (Havel & Goldfien, 1959) and by certain hormones, e.g. growth hormone (Raben & Hollenberg, 1958), catecholamines (Havel, 1959) and thyroid hormone (Rich, Bierman & Schwartz, 1959). Decreased lipid mobilization, and a reduction in the plasma FFA level, are brought about by increased glucose availability (Dole, 1956) and insulin (Dole, 1956; Gordon & Cherkes, 1956).

In starvation, where fat is the principal substrate and FFA levels are very high, insulin plays the primary role in regulating the pattern of fuel utilization (Cahill *et al.*, 1966). When starvation is com-

bined with trauma and allied conditions, catechol-amines may have a larger influence on substrate utilization. Evidence for increased fat oxidation is found in Moore's observation (Moore et al., 1952), derived from body compositional methods, that body fat is reduced to a greater extent after surgical trauma than for an equivalent caloric deficit alone. Wadstrom (1959) found significantly higher levels of FFA in the plasma 24 hr after cholecystectomy than could be accounted for by post-operative semi-starvation, and we have found increased levels of FFA to be associated with operation, multiple frac-tures, burns, peritonitis and shock. Lipid mobiliza-tion in this setting can be in excess of caloric needs and result in ectopic re-esterification of triglyceride. Fat infiltration of the liver and myocardium is ob-served after burns (Sevitt, 1962) and after multiple injuries complicated by peritonitis. Experimentally, catecholamines are capable of inducing fatty change in various tissues (MacKay, 1937; Aujard, 1953; Maling & Highman, 1958; Fiegelson et al., 1961).

Functional changes induced by excessive and calorically inappropriate lipid mobilization have not been fully defined. This study was undertaken to simulate the clinical situation of prolonged catechol-amine hypersecretion and to examine its effects on lipid content of non-adipose tissue, liver function, and the surface-active phospholipids of the lungs. Experimentally, catecholamines are known to be capable of causing tissue damage (Highman, Maling & Thompson, 1959; Schenk & Moss, 1966) but the mechanism is not clear. Tissue damage and inter-ference with organ function by catecholamines might occur either as a direct vascular effect, as a result of lipid infiltration of tissues, or by mobilization of fatty acids from structurally important tissue phospholipid.

Materials and methods

Thirteen dogs were used. One day before the experiment, using local anaesthesia, catheters were introduced into both external jugular veins. On the day of the experiment, with the animals awake and in a basal state, noradrenaline infusion was begun. One group of five animals were infused with nor-adrenaline for 24 hr, and a second group of five animals for 48 hr. Three control dogs received normal saline. The dose was selected to avoid short-term cardiovascular toxicity, e.g. cardiac failure (Gilbert & Hohf, 1964; Moss, Vittands & Schenk, 1966), hypotension from plasma volume reduction (Free-man, 1933; Schmutzer, Raschke & Maloney, 1961) and acidosis (Darby et al., 1960; Rosenthale & di Palma, 1962). In a preliminary study, administration at a rate of 1 μg/kg/min of noradrenaline base was found to produce lipid mobilization without toxicity. At the completion of the infusion the dogs were

anaesthetized with Diabutal and the lungs, heart, liver and portions of skeletal muscle were removed for histological examination and tissue lipid analysis.

Sequential blood samples were obtained for the measurement of FFA by the double extraction tech-nique (Dole & Meinertz, 1960), plasma insulin by radioimmunoassay (Yalow & Berson, 1960) and blood glucose (Hoffman, 1937). Serum glutamic oxaloacetic transaminase (SGOT) was measured in Henry units (upper limit of normal 35 units), and alkaline phosphatase (AP) in Bessey–Lowry units (upper limit of normal 2 units). Bromsulphthalein (BSP) excretion was measured at 40 min after in-jection of a dose of 5 mg/kg body weight.

The extractable surface activity of the lungs was assessed as follows. Twenty grams of wet lung tissue were minced, extracted with saline free of surface-active contaminants, and surface-tension–area curves recorded from the Wilhelmy surface balance. The surface was compressed cyclically to 20% of the maximum area; cycle time was 9 min. Good equi-librium was usually obtained after the first cycle; minimum and maximum surface tensions were taken from the mean of the second, third and fourth cycles.

Results

At the start of the infusion plasma FFA increased rapidly from control levels to the region of 3000 μEq/l, and remained elevated throughout the in-fusion period (Fig. 1). When the infusion was dis-
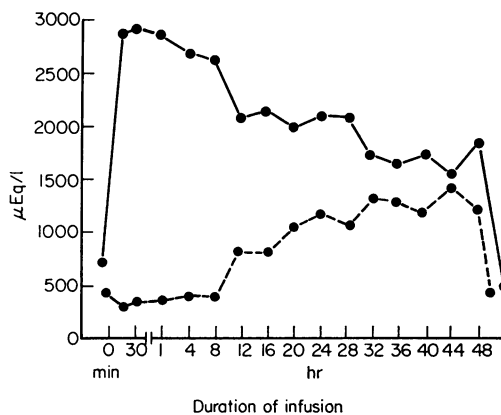

FIG. 1. Free fatty acid response to noradrenaline in-fusion. - - -, Control; ——, noradrenaline.

continued and anaesthesia induced the levels fell rapidly. The broken line shows FFA changes in the control group. In this group there was a progressive rise of plasma FFA level after 8 hr (presumably in part an effect of starvation) to moderately high levels by the end of the 48-hr period. Again there was

a marked fall in the level with the induction of anaesthesia.

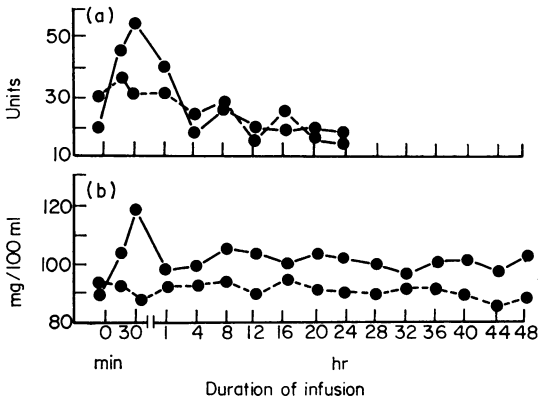Fig. 2 shows the responses of blood sugar and insulin. The initial rise in glucose and plasma insulin

FIG. 2. Insulin response (a) and glucose response (b) to noradrenaline infusion. Key as Fig. 1.

is not sustained, but both remain above control levels throughout the infusion period.
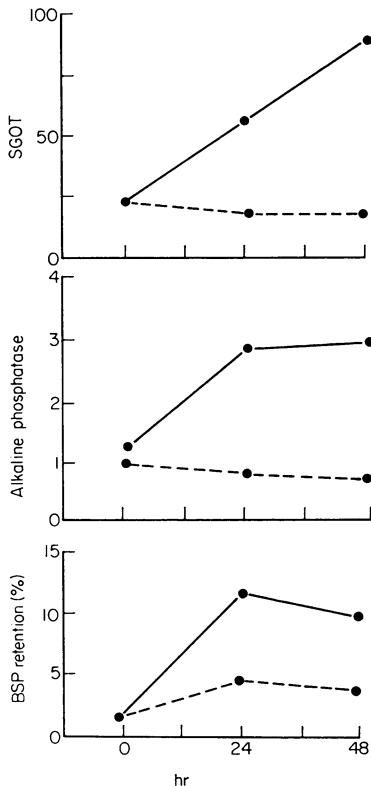
The level of SGOT was increased (Fig. 3) as was

FIG. 3. Noradrenaline infusion (1 μg/kg/min). Key as Fig. 1.

that of AP. There was moderate but not progressive impairment of BSP excretion.

The gross appearance of the liver in the dogs infused for 24 hr was very striking; it was yellow and cut like butter. Histologically there was marked intracellular deposition of triglyceride, evenly distributed throughout the lobules. Tissue lipid analysis confirmed that the increase in liver lipid was triglyceride, there being no significant alteration in either the phospholipid or cholesterol fractions. Other tissues showed marked fat deposition, particularly skeletal muscle and the heart.

Of special interest were the lungs. Lipid was present in the infusion group of animals but it was chiefly situated in the lining cells of the bronchi and bronchioles, in macrophages in the peribronchial areas and alveolar septal areas, and in alveolar lining cells. Lipid was not present in any great quantity, however, and certainly nothing like the amount present in liver and skeletal muscle. There was impairment of surface properties of lungs (increased minimum surface tension) in those animals infused for 48 hr. These changes are illustrated in Fig. 4.
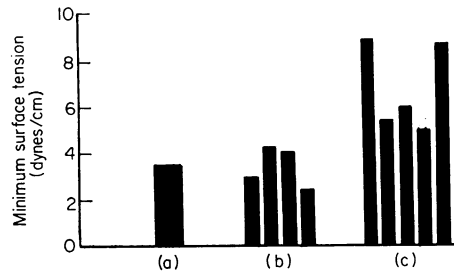

FIG. 4. Surface active properties of lungs. (a) Control, (b) 24-hr infusion group, and (c) 48-hr infusion group.

Normal value for minimum surface tension in dogs in our laboratory is 3.4 ± 0.7 dynes/cm. Lungs from animals in the 24-hr group were normal. In this group the minimum surface tension was 3.4 ± 0.9 dynes/cm. In the 48-hr group all results were abnormal, the mean value for minimum surface tension being 6.9 ± 1.8 dynes/cm ($P < 0.005$).

Discussion

Long-term noradrenaline infusion stimulated a marked increase in plasma FFA levels and an associated triglyceride deposition in tissues which do not normally contain much lipid, in particular liver and muscle. These changes were maximal at 24 hr. In the animals infused for 48 hr there was a diminution in the FFA response and tissue lipid deposition was much less obvious. In contrast, the observed enzyme changes increased progressively with time. Schenk, Galbraith & Moss (1966) noted a similar progressive increase of serum lactic dehydrogenase in dogs in-

fused with noradrenaline, an increase which was both time- and dose-dependent. It is difficult, therefore, to ascribe these enzyme changes, presumably reflecting cellular damage, to lipid deposition for although enzyme changes are more obvious at 48 hr lipid infiltration is not. An alternative possibility is that under catecholamine stimulation FFA are mobilized from structurally important phospholipid resulting in changes in cell membrane permeability and enzyme release, although there was no confirmatory evidence of this from estimation of the phospholipid in tissue extracts. It is unlikely that the enzyme changes could be the result of cell damage caused by ischaemia due to intense vasoconstriction. The haemodynamic effect of the dose used is small and probably insignificant (see Dr Hoffbrand's discussion, page 550). With regard to the effect of catecholamine infusion on liver function, both SGOT and AP were elevated suggesting liver damage, but not providing conclusive evidence of it, because both enzymes may arise from extrahepatic sources. BSP excretion was impaired and there appeared to be some correlation between this and the extent of hepatic lipid deposition.

It has been suggested that pulmonary lipid deposition associated with high blood levels of FFA is one of the factors contributing to the pulmonary lesion of shock and hypotension. However, in this experiment, although extravascular lipid was present it was scanty in amount and mainly situated in cells of the conducting airways, alveolar lining cells and alveolar macrophages. The likelihood of this small amount of lipid in this anatomical distribution interfering with pulmonary function is remote. It is possible however, that lipid mobilized from the lung interferes with surfactant production. Another relationship between hepatic lipid infiltration from increased lipid mobilization and fat embolism is the possibility of rupture of lipid-filled lakes into the hepatic venous sinusoids, with consequent embolization. In this experiment no intravascular fat emboli were seen on careful examination of the lungs and although liver triglyceride deposition was heavy, it was entirely intracellular in distribution.

Effects of long-term noradrenaline infusion on glucose and insulin response found in this study are at variance with the response to adrenaline described by Porte *et al.* (1966). In Fig. 4 it will be seen that at the start of the infusion there was a rise both in blood glucose and plasma insulin; Porte found that with adrenaline there was marked hyperglycaemia not associated with an insulin response; in fact, insulin levels remained low throughout the infusion period, rising only after the infusion was discontinued. It has been demonstrated by Allison and co-workers (Allison, Prowse & Chamberlain, 1967; Allison, Hinton & Chamberlain, 1968) that the insulin re-

sponse to intravenous glucose is impaired or absent in shock and trauma, including severe burns. It would appear in the light of this study that inhibition of insulin release is an adrenaline rather than a noradrenaline effect.

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