THE INHIBITION OF SUGAR TRANSPORT AND OXIDATION IN FAT CELL GHOSTS BY COLCHICINE

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SUMMARY: Colchicine inhibits glucose oxidation and the uptake of 2-deoxy-D-glucose in fat cell ghosts but has no effect on glucose oxidation by fat cell homogenates. This inhibition is rapid, reversible, and temperature-independent. Insulin-stimulated glucose oxidation and 2-deoxy-D-glucose transport are also inhibited by colchicine to an extent comparable to the basal processes.

Introduction:

Colchicine and other antimitotic drugs have been shown to inhibit the secretion of insulin from pancreas (1), catecholamines from adrenal medulla (2), histamine from human leukocytes (3) and from rat peritoneal mast cells (4), thyroid hormone from thyroid gland (5), norepinephrine and dopamine-β-hydroxylase from sympathetic nerves (6), and the release of free fatty acids from adipocytes (7). These effects in connection with the well-known property of colchicine and other antimitotic drugs to disrupt the microtubules prompted the speculation that the microtubular system might be involved in the secretory processes (8). More recent work has shown that colchicine is involved in a variety of other metabolic processes. For example, it affects the transport of nucleosides in several mammalian cell lines (9), inhibits the metabolism of glucose by isolated fat cells (10) and the insulin-stimulated metabolism of glucose to fatty acids (11), and inhibits the Mg^{++}-ATPase of synaptic membranes (12). In this study we demonstrate that colchicine inhibits the uptake of 2-deoxy-D-glucose and the oxidation

of glucose by fat cell ghosts but has no effect on the oxidation of glucose by fat cell homogenates.

Materials and Methods:

All radiochemicals were purchased from New England Nuclear. Crude bacterial collagenase was from Worthington Biochemical Corp. Bovine insulin, 2-deoxy-D-glucose and bovine serum albumin (fraction V) were obtained from Sigma. Colchicine was purchased from Calbiochem.

Animals used in all experiments were male Sprague-Dawley rats (175-200 grams) which had free access to food and water. The Krebs-Ringer bicarbonate buffer (KRB), pH 7.4, was used containing half the recommended amount of Ca^{++}, 3% bovine albumin (fraction V) and 0.5 mM glucose except if otherwise indicated. Isolated fat cells were prepared essentially according to the method of Rodbell (13) using however a lower concentration of collagenase. Specifically, epididymal fat pads were digested with collagenase at a concentration of 1.5 mg/ml in KRB buffer. Incubations were carried out for 30 to 45 min at 37° C in a metabolic shaker with a speed of 80 cycles per min. At the end of the incubation, the contents of incubation flasks were filtered through two layers of cheesecloth and then through one layer of Japanese silk without pressure. Cells were washed four times with warm KRB buffer that does not contain glucose. The ghosts were prepared by treating the isolated fat cells with hypotonic medium as described by Rodbell (14,15), except that it contained no ATP, NAD^{+} and $NADP^{+}$ (16). Fat cell homogenates were prepared in 0.25 M sucrose, as described by Zinman and Hollenberg (17); incubations were carried out as described by Potter (18). All incubations in this investigation were carried out in duplicate.

The glucose oxidation by ghosts was assayed as described by Rodbell (15) (see legend, Table 1). The uptake of 2-deoxy-D-[^{3}H] glucose by ghosts was assayed essentially by the method of Illiano and Cuatrecasas (16). Protein concentration was determined by the method of Lowry, et al (19).

Results:

Colchicine at concentration of 1 mM, has no effect on glucose oxidation by fat cell homogenates, but significantly inhibits the glucose oxidation by fat cell ghosts (Table 1). The inhibition of glucose oxidation by colchicine is reversible. As shown in Table 2, the treated ghosts were fully active after thorough washing to remove the colchicine in the medium. The poor responsiveness of the ghosts to insulin, which is apparant in Table 2, has also been observed by other investigators (15,16). The same experiment has been repeated with isolated fat cells.

Table 1. Effect of Colchicine on Glucose Oxidation by Fat Cell Ghosts

Colchicine (M)	Conversion of [1-^{14}C]Glucose into CO_2 (% of control)
-	100
10^{-5}	96
10^{-4}	91
10^{-3}	66
10^{-2}	28

Fat cell ghosts (101 µg of protein) were incubated for 2 h at 37° C in 1 ml KRB buffer containing 0.5 mM [1-^{14}C]glucose (0.1 µCi). Control value: 34.7 nmoles CO_2/mg protein/2 h.

After the pretreatment with colchicine, the cells' response to low concentration of insulin (25 µU/ml) is as good as that of the untreated cells (20). Insulin-stimulated glucose oxidation by fat cell ghosts is inhibited by colchicine to a similar extent (72%) to the basal inhibition (75%) (Fig. 1).

The uptake of 2-deoxy-D-[^{3}H]glucose by fat cell ghosts was studied in an attempt to determine the effect of colchicine on sugar transport. This glucose analog is transported by the same carrier system as the parent molecule and is phosphorylated but is not further metabolized (21,22,23). As shown in Fig. 2, both the basal and insulin-stimulated 2-deoxy-D-[^{3}H]glucose initial uptake were inhibited by colchicine to the same extent. The inhibitory effect on transport occurred at the earliest time point measured (2 min). The inhibition (44-47%) (Table 3) by colchicine of

Table 2. Reversibility of the Effect of Colchicine on Glucose Oxidation by Fat Cell Ghosts

Conditions	Conversion of [1-^{14}C]glucose into CO_2 (nmoles CO_2/mg protein/15 min)
Control ghosts	10.1
Control ghosts + insulin (1 mU/ml)	12.0
Treated ghosts*	10.5
Treated ghosts* + insulin (1 mU/ml)	12.4

* Fat cell ghosts (212 µg of protein) were pre-incubated with 10 mM colchicine (final volume 0.1 ml) at 37° C for 0.5 h. Cells were isolated by centrifugation (900 x g for 15 min) and washed three times with KRB buffer that does not contain glucose. All samples were incubated at 37° C for 15 min.

2-deoxy-D-glucose transport at 4° C is comparable to that observed at 25° C (Fig. 2).

Discussion:

In this study, we have demonstrated that colchicine at concentrations of 10 mM has little effect on glucose oxidation by fat cell homogenates. On the other hand, both the basal and insulin-stimulated glucose oxidation and the uptake of 2-deoxy-D-glucose by fat cell ghosts are significantly, and to comparable extents, inhibited by colchicine at this concentration. These results suggest that the inhibition of glucose metabolism by colchicine in isolated adipocytes observed previously (10,11), and fat cell ghosts, found in this investigation, is the result of a specific action on glucose transport across the plasma membrane.

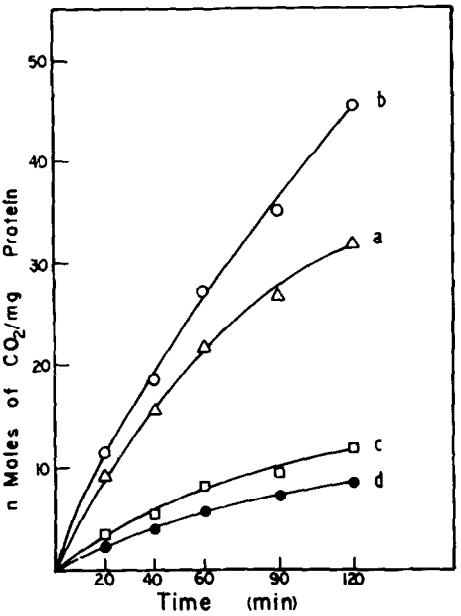

Fig. 1 Time course of basal and insulin-stimulated glucose oxidation by fat cell ghosts in the presence and absence of colchicine. The incubation mixture (see legend in Table 1) contains 105 µg of protein: (a) basal; (b) 1 mU/ml insulin; (c) 1 mU/ml insulin and 10 mM colchicine; (d) 10 mM colchicine.

The inhibition of glucose oxidation by colchicine is readily reversible by washing off the drug and the pretreated ghosts are still responsive to insulin. Apparently colchicine is not covalently bound to the membrane and the insulin receptors are not damaged on exposure to this drug.

The onset of the inhibitory effect of colchicine on transport is immediate (Fig. 2) not requiring pre-incubation of the fat cell ghosts with the drug. The percent inhibition in sugar transport by colchicine in fat cell ghosts at 4° C is comparable to that observed at 25° C. Microtubules are disrupted by either exposure to low temperatures (24,25) or after prolonged treatment with colchicine (26). Our results, in conjunction with the latter reports,

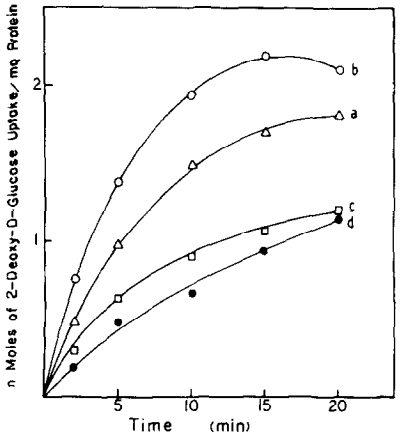

Fig. 2 Effect of colchicine on basal and insulin-stimulated 2-deoxy-D-glucose transport in fat cell ghosts.

Fat cell ghosts, about 150-200 µg of protein, were incubated for varying times at 25° C in 0.1 ml KRB buffer without glucose containing 1 mM 2-deoxy-D-[^{3}H]glucose (2 µc/0.1 ml), and other substances as indicated: (a) basal; (b) 1 mU/ml insulin; (c) 1 mU/ml insulin and 10 mM colchicine; (d) 10 mM colchicine.

Table 3. Effect of Colchicine on the Uptake of 2-Deoxy-D-[^{3}H]Glucose by Fat Cell Ghosts at 4° C

Time of Incubation (min)	Uptake of 2-Deoxy-D-[^{3}H]Glucose by Fat Cell Ghosts (cpm)		
	Control	Colchicine (10 mM)	% Inhibition
30	760	420	44
60	1200	640	47

The assay conditions were as described in Fig. 2 except that the incubations were performed at 4° C. The amount of fat cell ghosts used was 192 µg of protein per sample.

indicate that the action of colchicine is not related to microtubule disruption. If microtubules are present in the fat cell ghosts, an induction period would be anticipated for the inhibitory action of colchicine. Secondly, the percent inhibition at 25° C would be greater than that at 4° C, since at the latter temperature the microtubules would already have been disrupted in both the control and colchicine experiments.

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