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ETHANOLAMINE MIMICS INSULIN EFFECTS IN VITRO

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The comparative effects of insulin and ethanolamine on $^{14}CO_2$ production and lipid synthesis from [U-^{14}C]-D-glucose in isolated rat adipocytes were studied. Ethanolamine (10 mM) increased $^{14}CO_2$ production (glucose oxidation) about 5-fold and lipogenesis about 3-fold as compared to the control. Ethanolamine was more efficient than 25 μU/ml insulin regarding both parameters, but it was less efficient than 200 μU/ml insulin in glucose oxidation, and equally potent in lipogenesis. The combination of ethanolamine and insulin was more active than insulin alone. The mechanisms of ethanolamine action include facilitation of glucose transport and increase of pyruvate dehydrogenase activity.

Free ethanolamine is found in animal tissues and plasma in substantial amounts (1,2); however, its biological role is not completely elucidated. It was shown that ethanolamine inhibits azo-dye carcinogenesis (3) and is active in suppressing anaphylaxis and serotonin toxicity at low concentrations (4). The ethanolamine content in rat hepatoma and regenerating liver was found to be considerably higher than in the normal liver, and increased in parallel with the mitotic rate during liver regeneration (5). Ethanolamine was shown to be diuretic (6), to have antirachitic activity (7), to accelerate liver regeneration (8), to affect the hypothalamo-pituitary axis (9), to stimulate protein and nucleic acid synthesis (10), and to exhibit an overall anabolic effect (11). Ethanolamine was proved to be a growth-promoting factor in chickens (12) and yeasts (13). In relatively recent publications it was

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demonstrated that ethanolamine stimulates the growth of mammary carcinoma cells in culture (14), and is an essential component for the growth of hybridoma cells in serum-free medium (15). In short, ethanolamine is characterized as having broad-spectrum biological activity. The present investigation was generated by an earlier observation that ethanolamine potentiates glycogenolysis in muscles (16). In this report an in vitro insulin-like activity of ethanolamine in isolated rat adipocytes is described.

MATERIALS AND METHODS

Reagents - Collagenase (Type I) was purchased from Worthington Diagnostics (Freehold, N.J.), rat insulin from Novo (Copenhagen, Denmark), [U-^{14}C]-D-glucose from ICN (Irvine, CA), 2-deoxy-[U-^{14}C]-D-glucose from Amersham (Arlington Heights, IL), [1-^{14}C]pyruvate from New England Nuclear (Boston, Mass), ethanolamine hydrochloride, bovine serum albumin (BSA), EDTA, dithiothreitol, CoA, NAD, thiamine pyrophosphate and pyruvate from Sigma Chemical Co. (St. Louis, MO). Other reagents were of analytical grade.

Animals - Male Sprague-Dawley rats (300-350 g) were used for all experiments. The rats were kept in individual cages in a temperature- and light-controlled room (23°C, 12 h of light, 12 h of darkness), and had free access to Purina rat chow and water. The rats were sacrificed by cervical dislocation and the epididymal fat pads were quickly removed.

Preparation of isolated adipocytes - Isolated adipocytes were prepared by shaking pieces of fat pad with collagenase (3 mg/ml) in Krebs-Ringer bicarbonate buffer (pH=7.4) containing 30 mg/ml BSA, 0.1 mg/ml glucose and 4.7 mg/ml HEPES, using the method described by Rodbell (17). Adipocyte counts were performed in a hemocytometer.

In glucose oxidation and lipid synthesis studies the buffer was the same as for the isolation of adipocytes. For 2-deoxyglucose transport and pyruvate dehydrogenase studies, the buffer was prepared without glucose.

In all experiments about 2.5 x 10^5 adipocytes were incubated in a total volume of 1 ml. The incubations were carried out in a water bath (37°C) with shaking (80 osc/min) after gassing the samples with a mixture of 95% air and 5% CO_2.

Glucose oxidation - Glucose oxidation was measured by the production of $^{14}CO_2$ from [U-^{14}C]-D-glucose (17). The fat cells were incubated over a period of 2 hours in KRB buffer containing 0.1 μCi/ml [U-^{14}C]-D-glucose. The liberated $^{14}CO_2$ was collected on a piece of filter paper imbibed with 0.2 ml 25% phenylethylamine in methanol (18). The radioactivity was measured in a Beckman liquid scintillation counter.

Lipid synthesis - The incorporation of ^{14}C from [U-^{14}C]-D-glucose into the lipids of adipocytes was measured after a similar incubation as for the glucose oxidation. At the end of the incubation the adipocytes were separated by transferring the incubation medium into a Beckman microfuge tube containing silicone oil, and after centrifugation the lipids were extracted from the adipocytes with the Dole's extraction mixture (19) and counted.

Glucose transport - Isolated adipocytes were incubated with 2-deoxy [U-^{14}C]-D-glucose (0.31 μCi/ml) for 5 minutes preceded by one hour preincubation in KRB buffer (control) or KRB buffer containing insulin and/or ethanolamine.

Preliminary experiments with 5-, 10-, and 15-minute incubation periods have shown that the transport of 2-deoxy [U-^{14}C]-D-glucose into adipocytes had a linear pattern during that period of time.

Pyruvate dehydrogenase (EC 1,2,4,1) - The activity of this enzyme was measured according to the method of Taylor et al. (20). Adipocytes were preincubated for 30 minutes in KRB buffer with or without insulin and/or ethanolamine, then were homogenized and incubated with [I-^{14}C]pyruvate (0.18 μCi/ml) for 5 minutes.

Because of daily variations in total radioactivity counts the results are expressed as per cent of control samples (mean±SE). All measurements were made in triplicates. Statistically significant differences were calculated using Student's t test.

RESULTS

Figure 1 shows the effect of different concentrations of ethanolamine on glucose oxidation and lipogenesis. The

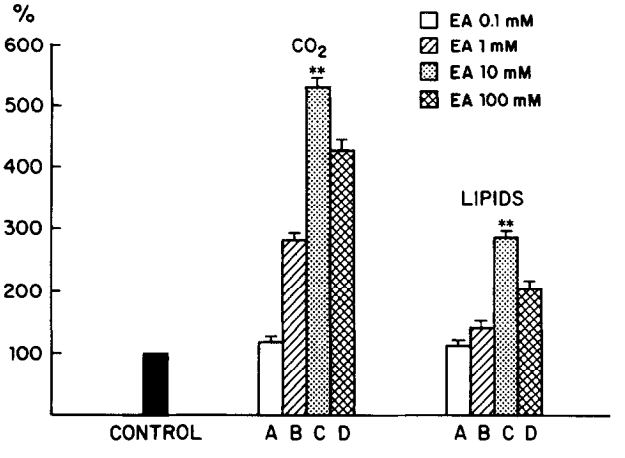

Figure 1. In vitro effects of different concentrations of ethanolamine (EA) on $^{14}CO_2$ production from [U-^{14}C]-D-glucose and on the incorporation of [U-^{14}C]-D-glucose into lipids in isolated rat adipocytes (n=6 for each value). The statistical significance for the columns C (compared to the columns B and D) is indicated by two asterisks which stand for $p<0.01$.

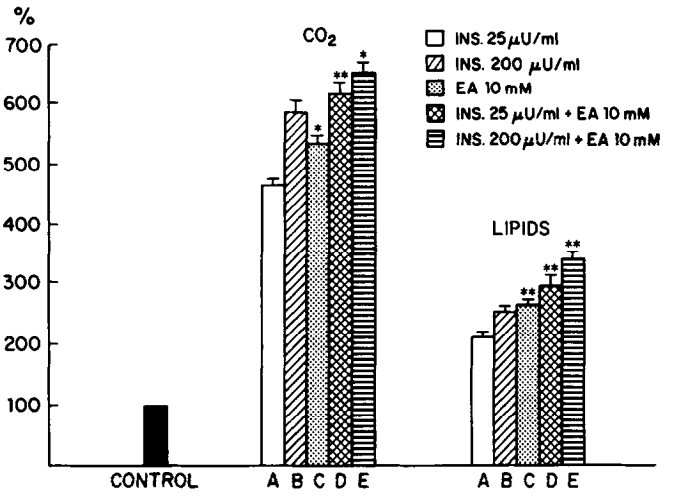

Figure 2. In vitro effects of insulin, ethanolamine (EA) and their combination on $^{14}CO_2$ production from [U-^{14}C]-D-glucose and on the incorporation of [U-^{14}C]-D-glucose into lipids in isolated rat adipocytes (n=6 for each value). The statistical significance is indicated by one or two asterisks which stand for $p<0.05$ and $p<0.01$ respectively (comparison of columns: C vs. A, D vs. A, E vs. B).

optimal concentration for both parameters was 10 mM which stimulated CO_2 production about 5 times and lipid synthesis about 3 times. It is noteworthy that in all experiments (including the controls) the radioactivity in lipids was 5-6 times higher than in CO_2.

In Figure 2 the relative effects of insulin and ethanolamine are depicted. In these and following experiments ethanolamine was used in its optimal concentration (10 mM). This concentration had a significantly higher promoting effect on both CO_2 production ($p<0.05$) and lipid synthesis ($p<0.01$) than 25 μU/ml insulin. However, ethanolamine had a lower ($p<0.05$) effect than 200 μU/ml insulin on CO_2 production, but was equally efficient in lipogenesis. The combination of ethanolamine and 25 μU/ml insulin had a higher effect on both CO_2 production ($p<0.01$) and lipid synthesis ($p<0.01$) compared to insulin alone. The same was true for the combination of ethanolamine and 200 μU/ml insulin.

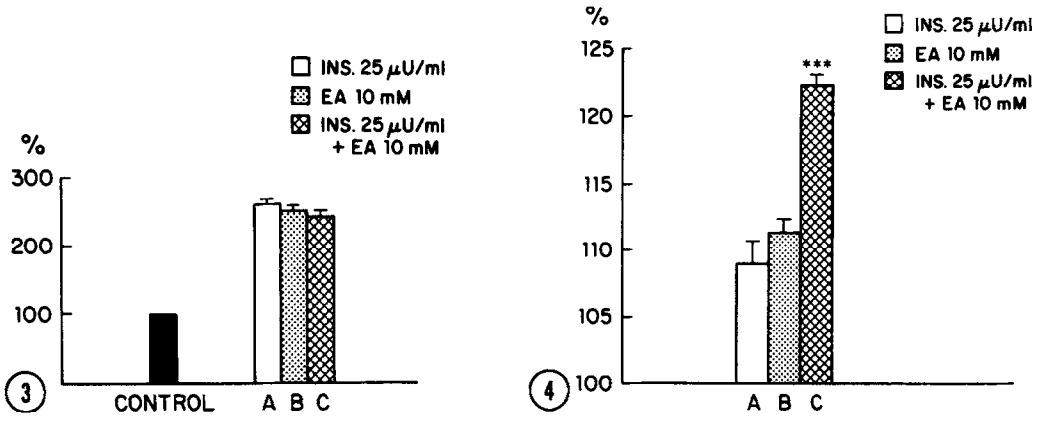

Figure 3. In vitro effects of insulin, ethanolamine (EA) and their combination on 2-deoxy[U-^{14}C]-D-glucose transport in isolated rat adipocytes. The differences between the columns A, B, and C are not statistically significant (n=6 for each value).

Figure 4. In vitro effects of insulin, ethanolamine (EA) and their combination on pyruvate dehydrogenase activity in isolated rat adipocytes as compared to the control (100%). The statistical significance for the column C (compared to the columns A and B) is represented by 3 asterisks which stand for $p<0.001$. The difference between the columns A and B is not statistically significant (n=6 for each value).

The transport of 2-deoxy-[1-^{14}C]-D-glucose into adipocytes is illustrated in Figure 3. Insulin (25 μU/ml) and ethanolamine (10 mM) alone and in combination had the same promoting effect on the transport of this sugar (about 2.5 times compared to the control).

The increase in pyruvate dehydrogenase activity (Figure 4) by insulin was not different from that when ethanolamine was present in the medium. However, their combination had a significantly ($p<0.001$) higher stimulatory action than each of them alone.

DISCUSSION

At the present time the mechanisms by which ethanolamine affects glucose oxidation and lipogenesis by such an unexpected degree is not completely understood. The optimal concentration of ethanolamine was somehow supraphysiological. However,

significant effects were also elicited by lower, almost physiological concentrations of this amine (Figure 1). It seems that ethanolamine induces its characteristic effects at least by two different mechanisms: facilitation of glucose transport and potentiation of pyruvate dehydrogenase activity. The transport effect is probably non-specific and could be related to changes in phospholipid content of cell membranes. The activation of pyruvate dehydrogenase could be the result of its dephosphorylation by ethanolamine (like insulin). In fact, ethanolamine was found to be active in phosphorylation-dephosphorylation mechanisms (21). It is also possible to speculate that the activity of this enzyme could be enhanced through the stimulation of the protein moiety, or through functional changes in the active site(s). In this context ethanolamine and its salts were shown to affect the activity of some enzymes: choline oxidase (22), alkaline phosphatase (23), ATP-ase (24), and GABA-transaminase (25).

It is interesting to note that the effects of insulin and ethanolamine on both glucose oxidation and lipogenesis are not additive, but somehow synergistic. This would suggest that the mechanisms of action of these two stimulators are not completely different. On the other hand, there was no additional effect of the combination of insulin and ethanolamine compared to insulin or ethanolamine alone on glucose transport. However, an additive effect of ethanolamine on insulin action was observed regarding pyruvate dehydrogenase activity.

In conclusion, despite unanswered questions, our results have shown an insulin-like activity of ethanolamine, and much remains to be done in the evaluation of the theoretical and practical values of this observation. Experiments are now in progress to examine the in vivo effects of ethanolamine on

glucose oxidation and lipogenesis with special reference to the glucose levels in the blood.

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