

Role of Glucose in Facilitating the Acute Steroidogenic Action of Adrenocorticotrophic Hormone (ACTH)¹ (34560)

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Despite the ubiquity of glucose as a substrate for metabolic processes, it has not unequivocally been shown to have a role in the series of biochemical reactions through which ACTH brings about an acute increase in adrenal steroid production. This acute action of ACTH is apparently mediated by cyclic AMP² (1) and probably involves the generation of a labile protein (2). It results in acceleration of the rate-limiting step in steroidogenesis, that at which cholesterol is converted to pregnenolone (3).

In 1956, Schönbaum *et al.* (4) reported that the ACTH-induced production of corticosterone by rat adrenal slices was impaired if glucose was excluded from the incubation medium. In 1962, however, Vance *et al.* (5) reported that they could find no difference between the rate of corticosterone formed by adrenal slices incubated with glucose and those incubated without. Since the role of glucose in adrenal cortical function is still controversial (6), we have reinvestigated the problem and have attempted to relate our observations to current concepts regarding the mechanism of action of ACTH.

Materials. The ACTH utilized in these studies was obtained from Parke, Davis Co. The incubation medium was Krebs-Ringer bicarbonate, pH 7.4, containing 0.5 % (w/v) bovine albumin and 10 mM glucose, unless otherwise indicated.

¹ These studies were supported in part by Grants-in-Aid 5-TO1-AM-05092, 5-RO1-AM-05318, and 4-K6-AM-3782 from the National Institute of Arthritis and Metabolic Diseases.

² The following abbreviations and trivial names are used: cyclic AMP = 3', 5'-adenosine monophosphate; NADP = nicotinamide-adenine dinucleotide phosphate (sodium salt); pregnenolone = 3 β -hydroxy-pregnen-5-ene-20-one.

Methods. Male Holtzman rats weighing between 180–220 g were hypophysectomized by the transaural approach. Two hr following hypophysectomy the adrenals were removed, trimmed free of fat, and quartered. One right and one left adrenal from different animals were placed in 2 ml of incubation medium and subjected to preliminary incubation for 60 min at 37° under an atmosphere of 95 % O₂ - 5 % CO₂ in a shaking water bath. The glands were then transferred to 2 ml of fresh medium, the test materials added, and the study period commenced. At the termination of the second incubation, the two adrenals were homogenized in the medium and the corticosterone content of the homogenate was determined by the method of Silber *et al.* (7).

In experiments involving simultaneous measurement of glucose oxidation and steroidogenesis, uniformly labeled ¹⁴C-glucose was added at the beginning of the second incubation. The ¹⁴CO₂ formed during 2 hr incubation was liberated by the addition of H₂SO₄ at the end of the incubation period and determined by the method of Fain *et al.* (8). The glands were homogenized with the acidified incubation medium and the corticosterone content of the homogenate was determined.

When cyclic AMP and corticosterone were to be measured simultaneously, six adrenals were subjected to preliminary incubation for 60 min. The glands were then transferred to 2 ml of fresh medium, ACTH was added, and the incubation was continued for periods ranging from 15 to 60 min. At the end of the second incubation, the glands were rapidly homogenized in 20 ml of 0.1 N HCl containing purified tritiated cyclic AMP (approximately 10,000 dpm). The acid homogenate

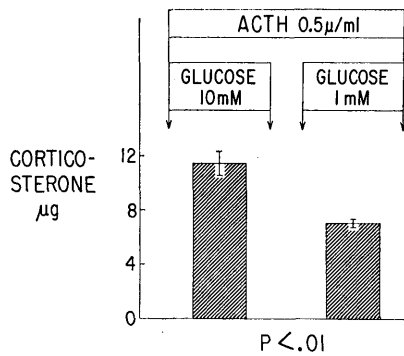


FIG. 1. Effect of glucose on ACTH-induced steroidogenesis. The amounts of corticosterone produced by two sliced adrenals during 2 hr incubation with 1 unit of ACTH are plotted. In this and all subsequent figures, unless otherwise specified, each bar represents the mean of four observations and the brackets indicate the standard errors of the means. Student's *t*-test was employed to determine *p* values.

was then fractionated and the cyclic AMP assayed by the method of Butcher *et al.* (9). The amount of corticosterone released into the incubation medium during each incubation period was determined.

Results and Comments. Lack of effect of glucose on corticosterone production in the absence of adrenal stimulators. In the absence of any steroidal precursors or any exogenous stimulators, adrenal production of corticosterone proceeded at a slow rate (approximately 1 μg of corticosterone/two adrenals/2 hr incubation) which was not influenced by glucose. It was important to utilize only adrenals from hypophysectomized rats, for adrenals obtained from intact animals formed more corticosterone than did those from hypophysectomized animals; and, under these circumstances, the rate of corticosterone formation was greater in the presence than in the absence of glucose.

Effect of glucose on ACTH-induced corticosterone production. The addition of ACTH to incubation media containing 10 mM glucose consistently resulted in marked increases in corticosterone production. The steroidogenic effect of ACTH was much less when it was added to media containing only 1 mM glucose (Fig. 1).

The poor response of the glucose-deprived adrenals was not due to irreversible injury to

the glands, since the addition of glucose to the medium after 2 hr of deprivation resulted in a distinct increase in responsiveness to ACTH (Fig. 2).

From these observations it was concluded that glucose deprivation impairs the acute steroidogenic response to ACTH, and further experiments were performed to determine where glucose exerted its effect in the sequence of events leading to corticosterone production.

Lack of effect of glucose on conversion of progesterone to corticosterone. Conceivably, the effect of glucose deprivation on steroidogenesis might have been to limit the efficiency of some step late in the pathway of corticosterone synthesis. To test this hypothesis, experiments were performed in which progesterone was added to the media, and its conversion to corticosterone was measured in the presence of various concentrations of glucose. Progesterone was converted to corticosterone equally well in the presence of either high or low concentrations of glucose in the media.

Effect of glucose on cyclic AMP-induced corticosterone production. It is currently

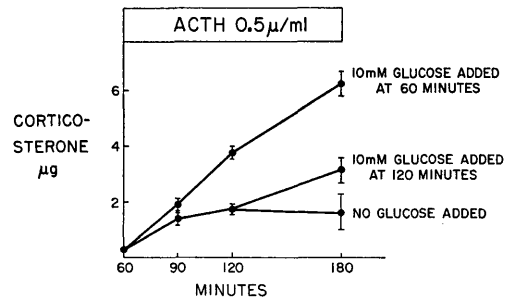


FIG. 2. Effect of glucose on adrenal responsiveness to ACTH. Rat adrenal slices were initially incubated for 1 hr in the absence of glucose. At the beginning of the second hr 1 unit of ACTH was added to all samples, which were then divided into three groups. The first group was incubated without glucose for an additional 2 hr (60–180 min on abscissa). The second group was incubated without added glucose during the second hour; then 10 mM glucose was added and the incubation was continued for a third hour. The third group was incubated in 10 mM glucose during both the second and third hours. The amounts of corticosterone produced by two sliced rat adrenals were plotted cumulatively.

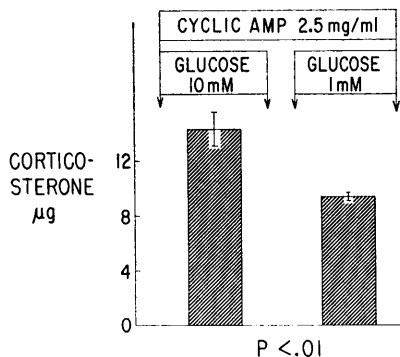


FIG. 3. Effect of glucose on cyclic AMP-induced steroidogenesis. The amounts of corticosterone produced by two sliced adrenals during 2 hr incubation are plotted.

thought that the effect of ACTH on the adrenal is mediated by cyclic AMP. Experiments were, therefore, performed to determine whether glucose facilitates the steroidogenic effect of cyclic AMP (Fig. 3). The addition of cyclic AMP to incubation media containing 10 mM glucose consistently resulted in marked increases in corticosterone production. The steroidogenic effect of cyclic AMP was greatly diminished by reduction of the glucose concentration to 1 mM. It was concluded that a major role of glucose in facilitating the action of ACTH was probably to facilitate the steroidogenic action of cyclic AMP.

Effect of glucose on ACTH-induced cyclic AMP formation by the adrenal. The fact that glucose facilitates the action of cyclic AMP does not necessarily imply that this is the only glucose-sensitive step in the sequence of reactions through which ACTH stimulates corticosterone production. Studies were also carried out to determine whether glucose might affect the process through which ACTH leads to an increase in the concentration of cyclic AMP in the adrenal gland. ACTH induced an increase in both cyclic AMP and corticosterone levels, but both effects were significantly greater ($p < .05$) in the presence of 10 mM glucose than in the absence of glucose (Fig. 4). It was concluded that glucose facilitates the steroidogenic action of ACTH in at least two ways: first, by supporting the action of ACTH in stimulat-

ing the formation of cyclic AMP by adrenal tissue, and second, by supporting the action of cyclic AMP in stimulating the conversion of cholesterol to steroids.

Lack of effect of glucose on NADPH-induced corticosterone production. Since NADPH has a stimulatory effect on corticosterone production *in vitro*, it has been suggested that the acute steroidogenic action of ACTH (and, therefore, of cyclic AMP) might entail the formation of NADPH (10). If this be true, then a role of glucose in acute steroidogenesis might be that of providing glucose-6-PO₄ or some other substrate which facilitates the conversion of NADP to NADPH. If glucose facilitates the formation of NADPH and has no major action subsequent to the step which is stimulated by NADPH, then exogenous NADPH added to adrenal tissue should be equally effective in stimulating steroidogenesis either in the

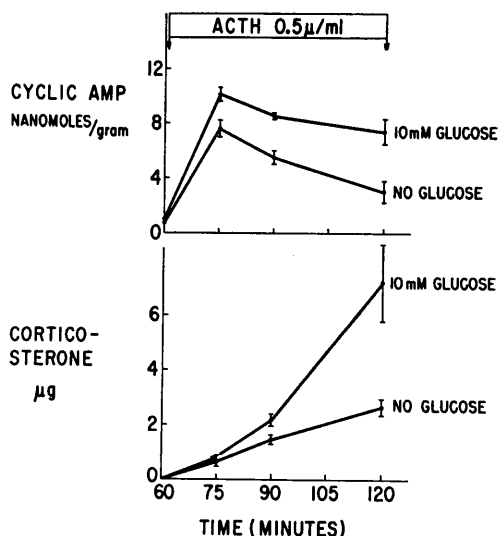


FIG. 4. Effect of glucose on ACTH-induced cyclic AMP formation and steroidogenesis. One group of rat adrenal slices was subjected to preliminary incubation for 60 min in glucose free-medium while a second group was incubated in medium containing 10 mM glucose. The medium of each group was then refreshed, ACTH was added to all samples, and the incubation continued. The cyclic AMP content of the glands determined at the times indicated and the cumulative quantities of corticosterone released into the medium during the total time intervals that elapsed after the addition of ACTH are plotted.

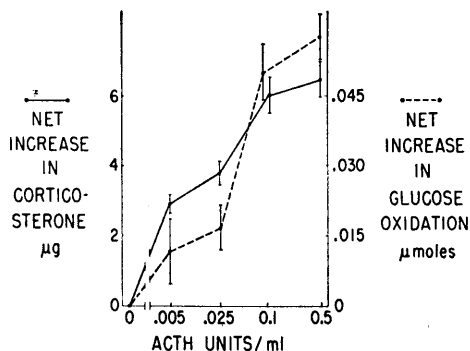


FIG. 5. Effects of graded doses of ACTH on steroidogenesis and glucose oxidation. The net amounts of corticosterone produced and glucose oxidized by two sliced adrenals during 2 hr incubation in response to graded doses of ACTH are plotted. "Net" amounts were calculated by subtracting the values observed in control incubations from those in which ACTH was added. Each point represents the mean of five observations.

presence or absence of glucose. Experiments were performed in which NADPH or an NADPH generating system (NADP together with glucose-6-phosphate) were added to adrenal tissues incubated with various concentrations of glucose. It was observed that NADPH and the NADPH generating system were as effective in stimulating corticosterone production in the presence of 1 mM glucose as in the presence of 10 mM glucose.

It was inferred that a role of glucose in facilitating the acute steroidogenic action of ACTH might possibly be to facilitate the formation of NADPH but that glucose was not essential for the subsequent steps in the acute steroidogenic process.

Effects of steroidogenic agents on glucose oxidation by the adrenal. If glucose does serve as a substrate in some biochemical reaction that is accelerated by ACTH, then one might ask whether glucose oxidation is stimulated by ACTH. The same question could be asked with reference to other steroidogenic agents. Accordingly, labeled glucose was added to the adrenal incubation media, and its conversion to labeled CO_2 was measured. It was observed that graded doses of either ACTH or cyclic AMP induced graded adrenal responses in terms of increased conversion of glucose to CO_2 (Figs. 5 and 6). In

contrast, NADPH (Fig. 7), the NADPH generating system and progesterone did not stimulate glucose oxidation by the adrenal. It thus appears that those agents that depend upon the presence of glucose for their maximum steroidogenic effectiveness are also those that stimulate glucose oxidation.

Corticosterone production and glucose oxidation in broken-cell adrenal preparations. Adrenal glands that had been homogenized were found to be unresponsive to ACTH or cyclic AMP either in terms of corticosterone production or glucose oxidation. Such preparations responded to NADPH with increased corticosterone production but without increased glucose oxidation (Fig. 8).

Discussion. The rate-limiting step in steroidogenesis is ordinarily considered to be the conversion of cholesterol to pregnenolone (3). Once formed, pregnenolone is readily converted to progesterone and then to corticosterone and other hormonal steroids. The rate-limiting step is accelerated by ACTH, which is thought to act by stimulating the formation of cyclic AMP, which is thought to stimulate the synthesis of a labile protein, which in turn stimulates a series of reactions culminating in the conversion of cholesterol to pregnenolone.

The present study has shown that at least two of these processes are glucose-sensitive. Glucose facilitates the formation of cyclic AMP in response to ACTH, and it facilitates the action of cyclic AMP. Thus, corticosterone production that is induced by ACTH or cyclic AMP can be diminished by depriv-

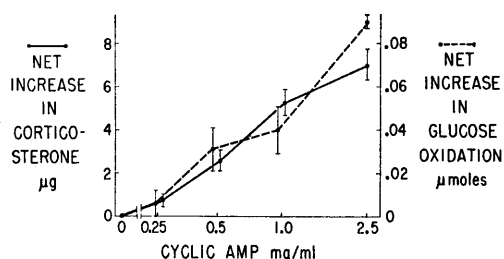


FIG. 6. Effects of graded doses of cyclic AMP on steroidogenesis and glucose oxidation. The net amounts of corticosterone produced and glucose oxidized by two sliced adrenals during 2 hr incubation in response to graded doses of cyclic AMP are plotted.

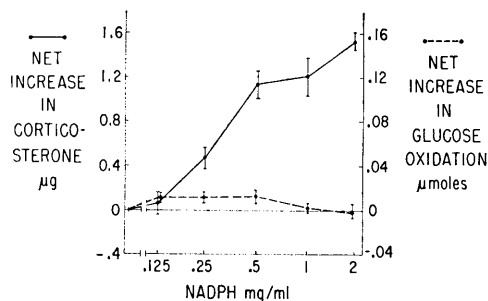


FIG. 7. Effects of NADPH on steroidogenesis and glucose oxidation. The net amounts of corticosterone produced and glucose oxidized by two sliced adrenals during 2 hr incubation in response to graded amounts of NADPH are plotted.

ing the adrenal of glucose, but corticosterone production induced by NADPH or progesterone is unimpaired by glucose deprivation.

Precisely what chemical reactions are facilitated by glucose is not clear at the present time. ATP is the substrate for adenyl cyclase in the formation of cyclic AMP, but it would be only speculative to assume that glucose increases the availability of ATP in the adrenal. The enzyme system involved in cholesterol side chain cleavage has been shown to require NADPH (11), and cholesterol cleavage is the step in the steroid pathway that is acutely accelerated by ACTH (3). It seems possible that the role of glucose in facilitating the action of cyclic AMP might be to provide a source of glucose-6-phosphate or some other substrate which is then utilized by the adrenal cortex in the conversion of NADP to NADPH.

Despite the uncertainty surrounding the

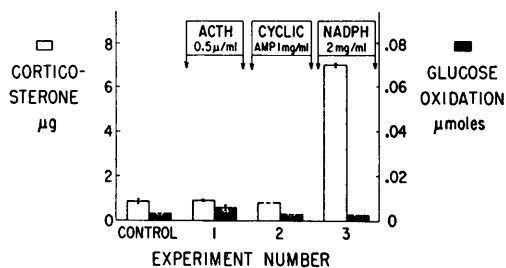


FIG. 8. Steroidogenesis and glucose oxidation in adrenal homogenates. The amounts of corticosterone produced and glucose oxidized by the equivalent of one adrenal during 2 hr incubation are plotted. Each bar represents the mean of three observations.

exact biochemical role of glucose in the adrenal, it seems that a theory that explains how glucose supports the action of ACTH and cyclic AMP should also take into account the fact that glucose oxidation is accelerated by these agents. That glucose oxidation is involved in the initiation of steroidogenesis rather than being an effect of high steroid levels is indicated by the fact that glucose oxidation is unaffected by the high levels of steroids formed in response to NADPH or progesterone.

The fact that glucose can support steroidogenesis *in vitro* does not eliminate the possibility that other substances might play a similar role. Schönbaum *et al.* (4, 12) found that pyruvate, adenosine, and uridine could also facilitate ACTH-induced steroidogenesis. Of all the substances used, however, only glucose was shown to be effective in the concentrations normally encountered in serum or extracellular fluid.

Summary. The possible role of glucose in facilitating the steroidogenic action of ACTH was investigated. In studies with quartered rat adrenals, reducing the glucose concentration from 10 to 1 mM significantly reduced the amount of cyclic AMP formed in response to ACTH and significantly reduced the amount of corticosterone formed in response to either ACTH or cyclic AMP but did not diminish the amount of corticosterone formed in response to exogenous NADPH or progesterone. In the presence of 10 mM glucose, stimulation of corticosterone synthesis with ACTH or cyclic AMP was accompanied by parallel increases in glucose oxidation, but stimulation of corticosterone synthesis by the addition of NADPH or progesterone was not accompanied by increased glucose oxidation. Adrenal homogenates failed to respond to ACTH or cyclic AMP with increases in corticosterone production or glucose oxidation; such preparations responded to NADPH with increases in corticosterone production but without increased glucose oxidation. One role of glucose in facilitating the steroidogenic action of ACTH might be to facilitate the formation of cyclic AMP, the intracellular mediator of the action of

ACTH. Since cyclic AMP-dependent steroidogenesis is facilitated by glucose and NADPH-dependent steroidogenesis is not, it is concluded that an additional role of glucose might be to serve as a substrate in some biochemical reaction that is accelerated by cyclic AMP, possibly the generation of compartmentalized NADPH.

We wish to acknowledge the advice and collaboration of Dr. R. W. Butcher and the excellent technical assistance of Christine E. Baird in performing the assays for cyclic AMP.

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Received July 31, 1969. P.S.E.B.M., 1970, Vol. 133.