Release of ACTH from Isolated Pituitary Cells: an Energy Dependent Process[1] (36561)

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Although the secretion of protein is generally considered to be an energy dependent process (2), relatively little is known regarding the energy requirements of adenohypophysial hormone release. To learn more about this process, we have studied the effect of various maneuvers designed to alter energy metabolism on spontaneous and induced release of ACTH from isolated pituitary cells.

Materials and Methods. Pituitary cells were dispersed from the anterior lobes of male Sprague-Dawley rats by a combination of trypsin and mechanical agitation; the cells were collected by centrifugation and resuspended to a concentration of 1 pituitary equiv./ml in Krebs–Ringer bicarbonate (KRB) buffer containing 0.2% glucose (except as indicated below), 0.5% bovine serum albumin (KRBGA) and 32 mg lima bean trypsin inhibitor, at pH 7.4, as previously described (3). Incubates were prepared by pipetting 0.9 ml aliquots of cell suspension into 10 ml Teflon beakers; in most experiments 8 to 12 incubates were prepared and in all cases the number of incubates equaled the number of pituitaries trypsinized.

Preincubation. Isolated pituitary cells were preincubated at 37° for 10 min in a Dubnoff shaker under 95% O_2:5% CO_2.

Incubation. Additions appropriate for determining spontaneous or induced ACTH release were made as follows: spontaneous release, 0.1 ml of appropriate vehicle; induced release, 0.1 ml of rat hypothalamic median eminence (HME) extract (1 HME/ml), arginine vasopressin (1 unit/ml), or *N*-ethylmaleimide (NEM), (2.5 m*M*). Incubations were carried out for 40 min under one of the following conditions: Standard condition (KRBGA, 37°, 95% O_2:5% CO_2) cold (KRBGA, 2°, air); anoxia (KRBGA, 37°, 95% N_2:5% CO_2); glucose free [KRBA (no glucose), 37°, 95% O_2:5% CO_2]. To determine the effect of metabolic inhibitors on spontaneous and induced ACTH release the following drugs were added prior to preincubation, at the final concentrations indicated, and the cells were incubated for 40 min under standard conditions: 2,4-dinitrophenol (DNP), 0.25 m*M*; iodoacetate, 2.5 m*M*; oligomycin, 1 μg/ml; antimycin A, 0.01 m*M*.

Postincubation. In order to eliminate possible artifacts due to direct effects of substances tested for an action on ACTH release on the subsequent bioassay for ACTH, all additions made prior to or during incubation were "crossed" at its termination, *i.e.*, after incubation substances tested for an action on ACTH release (HME, drugs, etc.) were added to controls and vehicle to experimentals. The suspensions were transferred to centrifuge tubes, the cells removed by centrifugation (600 g/5 min) and the separated medium recentrifuged (1500 g/3 min). The medium was acidified to pH 3.5 with HCl, heated to 100° for 5 min, appropriately diluted, and assayed for ACTH according to the isolated adrenal cell technic of Sayers, Swallow and Giordano (4). In every case 3 doses of medium and 6 or more doses of USP Standard ACTH (Third International Standard, 1.5 intravenous units/vial) were used, and each dose was given in duplicate.

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TABLE I. Protocol and Results of Typical Experiment on Effect of Metabolic Inhibitor on ACTH Release from Isolated Pituitary Cells.[a]

Preincubation, 10 min			Incubation, 40 min		Postincubation[f]				ACTH
Incubate no.	Ant. A[b] (ml)	Veh. 1[c] (ml)	HME[d] (ml)	Veh. 2[e] (ml)	Ant. A (ml)	Veh. 1 (ml)	HME (ml)	Veh. 2 (ml)	(μU/ml; mean ± SD)[g]
1	—	0.01	—	0.1	0.01	—	0.1	—	96 ± 12
2	—	0.01	—	0.1	0.01	—	0.1	—	100 ± 12
3	—	0.01	0.1	—	0.01	—	—	0.1	226 ± 28
4	—	0.01	0.1	—	0.01	—	—	0.1	240 ± 21
5	0.01	—	—	0.1	—	0.01	0.1	—	104 ± 15
6	0.01	—	—	0.1	—	0.01	0.1	—	97 ± 15
7	0.01	—	0.1	—	—	0.01	—	0.1	103 ± 12
8	0.01	—	0.1	—	—	0.01	—	0.1	102 ± 13

[a] Isolated pituitary cells were prepared as described in Materials and Methods; incubations were conducted under standard conditions: KRBGA, 37°, 95%O_2:5%CO_2.

[b] Antimycin A, 1 m*M*, in vehicle 1 (acetone); 0.01 ml added to beakers and solvent evaporated prior to addition of cells.

[c] Vehicle 1, acetone, 0.01 ml added to beakers and evaporated prior to addition of cells.

[d] HCl extract of rat HME, neutralized with NaOH and diluted to 1 HME/ml in KRBGA.

[e] Vehicle 2, HCl neutralized with NaOH and diluted in KRBGA as in footnote *d*.

[f] Postincubation additions were made to centrifuge tubes used for separation of cells from medium; volatile solvents were allowed to evaporate prior to introduction of the incubates.

[g] ACTH content of incubation medium separated from the cells and prepared for bioassay as described in Materials and Methods; SD is standard deviation of ACTH bioassay.

Extracts of rat HME were prepared by homogenizing freshly excised tissue in 0.1 *N* HCl. Insoluble material was removed by centrifugation (16,000 rpm/30 min, Sorvall SS 34 rotor) and twice reextracted with 0.1 *N* HCl. Prior to addition to the incubates, the extracts were adjusted to pH 7.0 with NaOH and appropriately diluted in KRBGA. Arginine vasopressin, 320 units/mg [pressor assay (5)], in 0.1 *M* acetic acid was adjusted to pH 7.0 with NaOH and appropriately diluted in KRBGA before addition to the incubates.

Results. The protocol and results of a typical experiment designed to test the effect of metabolic inhibitor on ACTH release are shown in Table I. Isolated pituitary cells incubated under standard conditions spontaneously released small but significant amounts of ACTH (Table I, 1 and 2); 0.1 HME induced the release of additional hormone (Table I, 3 and 4). In the presence of 0.01 m*M* antimycin A, spontaneous release of ACTH was not affected (Table I, 5 and 6), however, HME induced release of the hormone was abolished (Table I, 7 and 8).

Figure 1 summarizes the results of a number of experiments showing the effect of metabolic inhibitors as well as other maneuvers designed to alter energy metabolism on spontaneous and HME induced ACTH release. In each experiment the release of ACTH, spontaneous or induced, was expressed as the percentage of spontaneous release under standard incubation conditions (KRBGA, 37°, 95% O_2:5% CO_2), determined in the same experiment. The spontaneous release of ACTH (open bars) was increased when the cells were incubated at 2° but not significantly changed by any of the other maneuvers designed to alter energy metabolism (anoxia, glucose removal, or the addition of DNP, iodoacetate, oligomycin, or antimycin A). HME induced release of ACTH (shaded bars) was decreased by glucose removal, DNP, and iodoacetate and abolished by cold, anoxia, oligomycin and antimycin A. While we have not studied vasopressin induced release of ACTH under all of the conditions indicated above, we have observed that glucose removal, oligomycin and antimycin A inhibit vasopressin stimulated release of the

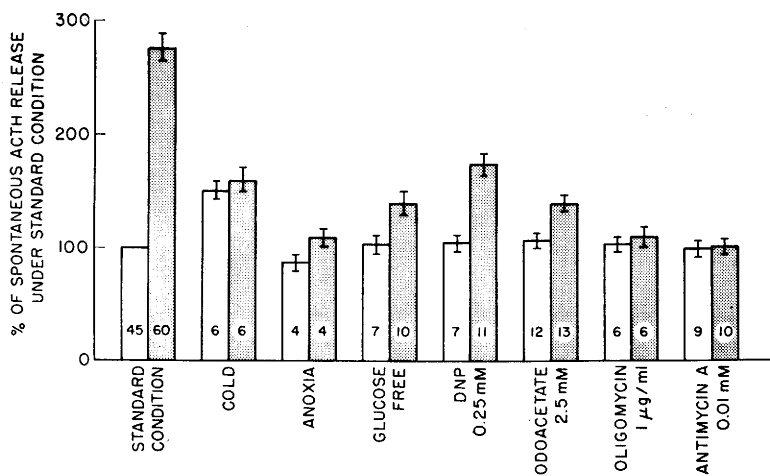

FIG. 1. Effect of antagonists of energy metabolism on spontaneous and HME induced ACTH release. Isolated pituitary cells were incubated under conditions noted on abscissa, in the absence (open bars) or presence (shaded bars) of 0.1 HME; number of incubations given by figure in bars; vertical line is standard error of the mean.

hormone (Fig. 2).

In contrast to the metabolic inhibitors (DNP, iodoacetate, oligomycin and antimycin A) the sulfhydryl reagent NEM, significantly increased ACTH release in each of 9 incubations. However, unlike HME or vasopressin induced release of ACTH, NEM stimulated release of the hormone was not decreased by the metabolic inhibitors antimycin A or oligomycin. A typical experiment illustrating these effects is shown in Fig. 3.

Discussion. The secretion of protein is generally considered to be an energy dependent process (2). However, in the case of the adenohypophysis little information regarding energy requirements is available and much of

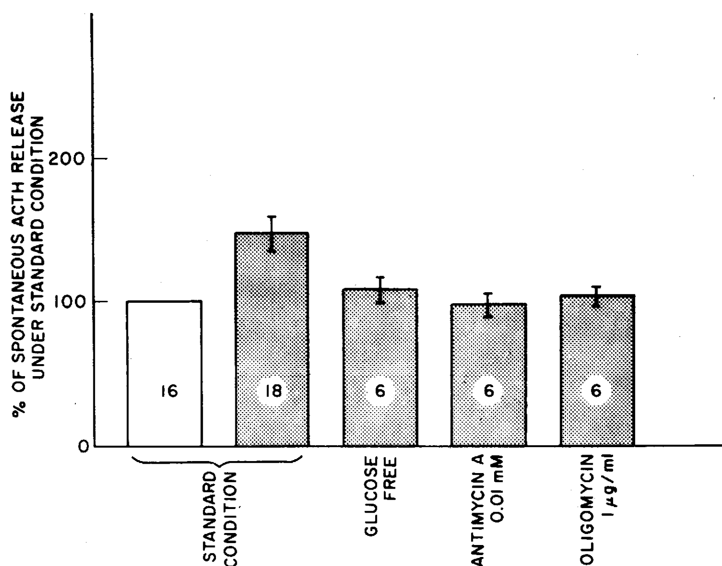

FIG. 2. Effect of antagonists of energy metabolism on vasopressin induced ACTH release. Incubations conducted under conditions noted on abscissa, in the absence (open bars) or presence (shaded bars) of 100 mU vasopressin.

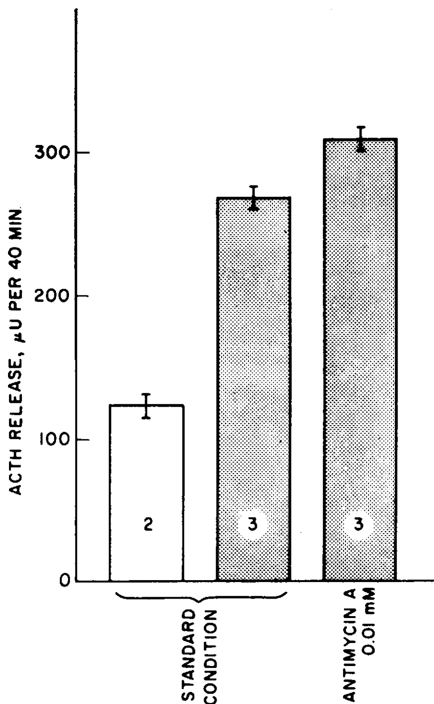

FIG. 3. Effect of antimycin A on NEM induced ACTH release. Incubations conducted under conditions noted on abscissa, in the absence (open bar) or presence (shaded bars) of 0.25 m*M* NEM.

that is conflicting. Samili and Gerschwind (6) reported that LH release *in vitro* was partially inhibited by oligomycin but not by DNP or cyanide. Wilber and Utiger (7) found that TSH release was inhibited by oligomycin or DNP. Guillemin [see (8)] confirmed the inhibitory action of oligomycin on TSH release, but found that DNP and also cyanide were without effect. Bowers, Lee and Schally (9) reported that TSH release *in vivo* was inhibited by oligomycin but not DNP. With regard to ACTH release, Chan, DeWied and Saffran (10) noted in single experiments that vasopressin induced release of the hormone *in vitro* was decreased by omission of oxygen or glucose.

The present study indicates that HME and vasopressin stimulated release of ACTH from isolated pituitary cells is an energy dependent process. As in other systems in which energy metabolism has been found necessary for protein secretion a number of possibilities for explaining this dependence exist: (a) Continued *de novo* synthesis of pituitary hormones may be a necessary condition for release. Although the bulk of the experimental evidence appears to indicate that such is not the case [for a recent review see (8)], there have been a few instances in which release of ACTH (11, 12) as well as other pituitary hormones (13, 14) have been reported to be decreased by inhibitors of protein or RNA synthesis. (b) Energy metabolism may be required for the occurrence of various "membrane phenomena" associated with transport and/or release of the hormones. In the case of protein secretion from pancreatic acinar cells, Jamieson and Palade (15) have suggested that the repeated fission–fusion of membranes required for transport between the transitional elements of the rough endoplasmic reticulum and the small, smooth-surfaced vesicles at the periphery of the golgi complex is probably the energy requiring step. (c) Energy may be required for the production of an intermediate necessary for hormone release. One obvious candidate for such an intermediate is cyclic-AMP which has been implicated in the release of several pituitary hormones (16, 17) including ACTH (18).

In contrast to HME and vasopressin stimulated release of ACTH, spontaneous release of the hormone is not inhibited by antagonists of energy metabolism. This finding could be explained by postulating that sufficient energy metabolism for spontaneous (but not stimulated) ACTH release remained under the conditions employed; alternatively, under the conditions of our experiments the mechanisms responsible for spontaneous and stimulated ACTH release may be different. Adequate data for deciding between these possibilities is not available. However, we are currently inclined toward the latter since time studies have shown that spontaneous release of ACTH from isolated pituitary cells occurs abruptly and terminates at/or prior to 5 min of incubation (indicating that it is perhaps due to leakage from a small number of damaged or broken cells) whereas HME stimulated release of the hormone continues at approximately a constant rate for up to 60 min of incubation (19). The ability of cold

(2°) to stimulate the release of ACTH from isolated pituitary cells is reminiscent of the findings of Douglas, Ishida and Poisner (20) that lowering the incubation temperature below 15° increased the release of vasopressin from posterior pituitaries *in vitro*. Furthermore, the stimulatory effect of cold on ACTH release and also on vasopressin release (20) is apparently not due to a decrease in the availability of metabolic energy required for storage of the hormones, since other maneuvers designed to inhibit energy metabolism did not augment spontaneous release. The elucidation of the mechanism whereby cold brings about release of ACTH must await further investigation.

Unlike the metabolic inhibitors (DNP, iodoacetate, oligomycin, and antimycin A) which did not alter spontaneous ACTH release, the sulfhydral reagent NEM significantly increased release of the hormone. This finding is again similar to the finding of Douglas, Ishida and Poisner (20) that sulfhydral reagents increase the release of vasopressin from posterior pituitaries and also of D'Iorio (21) that these agents release catecholamines from chromaffin granules. Although the basis for this effect is unknown, it is perhaps significant that NEM induced ACTH release is not dependent on metabolic energy as is HME and vasopressin induced release of the hormone, a finding which may be of use in differentiating CRF (or CRF-like substances) from nonspecific releasers of ACTH.

Summary. The influence of various maneuvers designed to alter energy metabolism on spontaneous and induced release of ACTH from isolated pituitary cells was studied. Spontaneous release was increased by cold (2°) but not significantly changed by anoxia, glucose removal, DNP, iodoacetate, oligomycin, or antimycin A. On the other hand, induced release of ACTH was inhibited by all of the maneuvers tested. These findings indicate that induced release of ACTH from isolated pituitary cells is an energy dependent process.

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