The responsiveness to (1–24) ACTH of Mongolian gerbil adrenals superfused in vitro as a function of basal secretion

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Summary. Wide variations have been found in the responsiveness to (1–24)ACTH of Mongolian gerbil adrenal glands superfused in vitro. These variations were not correlated to corticosteroid plasma levels or to adrenal weights. In contrast, (1–24)ACTH-stimulated secretion greatly depended on basal in vitro secretion (quarters: $r = 0.97$, $p < 0.01$; slices: $r = 0.90$, $p < 0.001$) indicating that the fine adjustment of basal corticosteroidogenesis forms an important part of the regulatory mechanisms modulating (1–24)ACTH-stimulated corticosteroid secretion in vitro.
Key words. Adrenals; in vitro superfusion; corticosteroids; (1–24)ACTH; Mongolian gerbils.

The production of corticosteroids by in vitro superfusion of adrenal glands follows a reproducible pattern that is characteristic of the species used, the physiological status of the animals, and the time of day the glands were removed[1–13]. However, a common finding in these experiments was the wide variation of basal corticosteroid secretion and the relative insensitivity to ACTH in vitro[2, 3, 5–8, 11]. This comparative insensitivity suggests that it is only with difficulty that ACTH penetrates to its receptor sites, especially in relatively thick tissue blocks, and that the permeability of ACTH through the tissue mass itself may influence the characteristics of the cell response. In order to test whether the size of tissue pieces may have a significant influence on the activity of the adrenal gland superfused in vitro, we have investigated the variability of both basal and (1–24)ACTH- stimulated corticosteroid secretion by adrenal quarters and slices from individual animals, and we have attempted to relate the responsiveness of superfused tissue to (1–24)ACTH to the basal secretion of corticosteroids.

Material and methods. Adult male Mongolian gerbils (*Meriones unguiculatus*, 8–10 months of age) and tree shrews (*Tupaia belangeri*, 2–6 years of age) from our animal colony were used. They were maintained singly under controlled temperature, relative humidity and lighting conditions (Mongolian gerbil: 22 ± 1°C; 55%; 02.00–14.00 h; tree shrew: 24 ± 1°C; 50–60%; 11.00–23.00 h). Food and tap water were available ad libitum.

Superfusion. Within 30 s after decapitation of the animals and collection of blood from the trunk, adrenals were removed, dissected free of fat and adhering tissue and cut into quarters or 300-µm slices with a McIlwain tissue chopper (The Mickle Laboratory Engineering Co., Mill Works, England). Tissue was then transferred into superfusion flasks, maintained at 36°C and superfused with medium (Krebs-Ringer bicarbonate, plus 1.2 mmol/l glucose) at a flow rate of 0.7 ml/min. To allow the cells to reach steady-state conditions[11], adrenal pairs of individual animals were superfused with medium alone for 60 min before (1–24)ACTH (Acortan simplex®, Ferring, Kiel; each ampoule contained 30 IU (1–24)ACTH, 50 mg glucose, 5 mg phenol) was added to the medium (0.01–1.0 IU/ml). At 15- or 30-min intervals, 5-min samples were collected, during a period of 120–180 min. All tests began between 10.30 and 11.00 h.

Radioimmunoassay of corticosteroids. Corticosteroid plasma levels were determined by radioimmunoassay after extraction (5 µl plasma, 3.0 ml dichlormethane), corticosteroid amounts in superfusates by direct RIA. The corticosteroid antiserum was raised against cortisol-21-hemisuccinyl bovine serum albumin (courtesy Prof. F. Bidlingmaier, Department of Clinical Biochemistry, University of Bonn, Bonn, FRG). This antiserum cross-reacted with cortisol (100%), cortisone (100%), corticosterone (100%), 11-deoxycortisol (100%), 11-deoxycorticosterone (100%), aldosterone (1%), 17-hydroxyprogesterone (95%) and testosterone (8%). Since levels of aldosterone, 17-hydroxyprogesterone and testosterone in superfusates of Mongolian gerbil adrenals are very low[9, 11, 12], mainly glucocorticosteroids were measured with this antiserum. The sensitivity of the assay was 20 pg/tube, the coefficient of variation within assays ranged from 10.9 to 1.4% ($n = 10$) and between assays from 10.5 to 6.7% ($n = 10$).

Statistics. The significance of the observed differences was calculated using the Mann-Whitney U-test (independent groups) or the Wilcoxon matched pairs signed rank test (dependent groups). Values are given as means ± SE. Differences were considered to be not significant if the calculated values exceeded the 5%- probability value. Correlation coefficents were calculated using standard regression analyses.

Results. Corticosteroid secretion by adrenal quarters superfused in vitro decreased significantly during the first 60 min of superfusion (30 min: 4.67 ± 1.73, 60 min: 3.82 ± 1.70 ng/adrenal pair/min, $n = 27$, $p < 0.01$; table 1).While the presence of 0.01 IU/ml (1–24)ACTH did not stimulate corticosteroid release, addition of 0.1 or 1.0 IU/ml (1–24)ACTH caused a significant stimulation of corticosteroid secretion (table 1). However, as in other systems for studying adrenal function, there was considerable variation between individual experiments in basal and (1–24)ACTH- stimulated corticosteroid secretion which could not be correlated to corresponding plasma levels ($r = -0.14$, $n = 96$, $p > 0.05$) or to the weight of tissue quarters ($r = 0.11$, $n = 96$, $p > 0.05$; data not shown). On the other hand, adrenal response to (1–24)ACTH was positively correlated to basal corticosteroid release in vitro (0.1 IU/ml: $r = 0.55$, $n = 11$, not significant; 1.0 IU/ml: $r = 0.97$, $n = 8$, $p < 0.01$).

In another set of experiments it was tested whether the large variation of basal and (1–24)ACTH- stimulated corticosteroid release was mainly due to variations in the number of cells/flask which were in contact with medium or (1–24)ACTH during superfusion, or was dependent on the interindividual differences in the secretory activity of cells superfused in vitro. For this purpose, adrenal glands of individual animals were cut into uniform, 300-µm-thin slices and were superfused with medium without or with 0.1 IU/ml (1–24)ACTH over 240 min. As summarized in table 2, corticosteroid release significantly decreased during the first 60 min of superfusion ($p < 0.001$). In the presence of (1–24)ACTH, a significant stimulation of secretion was observed at 90–240 min of superfusion (90–210 min: $p < 0.001$, 240 min: $p < 0.01$). Similarly to the results for quarters, maximum secretion during (1–24)ACTH stimulation was positively correlated to basal secretion (30 min: $r = 0.90$, 60 min: $r = 0.98$, $p < 0.001$; table 2).

Discussion. Over the last decade, the secretory activity of the adrenal gland has been extensively studied using cell suspensions obtained by enzymatic digestion and mechanical dis-

Table 1. Corticosteroid secretion by Mongolian gerbil adrenal quarters superfused in vitro: relationship between basal secretion and maximum output during (1–24)ACTH stimulation. After adrenal quarters had been superfused with medium for 60 min, (1–24)ACTH (0.01–1.0 IU/ml) was added to superfusion medium over 120 min and 5-min samples were collected at 15-min intervals. * $p < 0.05$, ** $p < 0.005$ (Wilcoxon test).

Experiment No.	Corticosteroid secretion (ng/adrenal pair/min) Length of superfusion (min) 15	30	45	60	75	90	105	120	135	150	165	180
0.0 IU/ml (1–24)ACTH												
1	6.36	6.05	5.50	5.17	5.00	5.06	5.92	6.40	5.84	5.80	5.87	5.62
2	8.73	4.88	3.70	3.43	3.32	3.33	3.50	3.39	3.23	3.35	3.33	3.34
3	4.84	4.40	4.15	3.90	4.07	3.91	3.36	3.18	2.83	2.52	2.84	2.44
4	6.60	4.61	4.45	4.16	4.13	4.10	4.26	4.32	3.97	3.89	3.95	3.80
Mean	6.63	5.04	4.45	4.17	4.13	4.10	4.26	4.32	3.97	3.89	4.00	3.80
SE	1.60	0.69	0.76	0.74	0.69	0.72	1.20	1.47	1.33	1.39	1.33	1.34
0.01 IU/ml (1–24)ACTH												
1	3.39	2.61	2.19	1.93	2.07	2.29	2.39	2.49	2.25	2.41	2.44	2.31
2	4.62	4.43	3.87	3.22	2.51	2.53	2.43	2.97	2.90	2.90	2.81	2.79
3	3.49	3.24	2.99	2.25	2.25	2.94	3.19	3.28	3.09	3.20	4.05	3.53
4	3.83	3.43	3.02	2.47	2.28	2.59	2.67	2.91	3.56	3.23	3.39	2.92
Mean	3.83	3.43	3.02	2.47	2.28	2.59	2.67	2.91	2.95	2.94	3.17	2.89
SE	0.56	0.75	0.69	0.55	0.18	0.27	0.37	0.33	0.54	0.38	0.70	0.50
0.1 IU/ml (1–24)ACTH												
1	6.34	4.79	3.98	5.06	5.06	5.25	6.72	7.08	6.58	5.96	4.84	4.82
2	4.03	4.32	3.96	3.57	5.77	5.14	7.73	6.97	7.28	6.68	7.61	7.37
3	3.40	2.97	2.47	2.21	2.92	4.35	4.88	5.18	6.71	4.99	4.27	4.08
4	5.22	4.40	4.34	4.72	4.93	5.11	5.09	5.13	5.14	5.08	4.94	4.68
5	6.44	6.27	5.21	5.04	5.51	5.49	5.55	5.88	5.66	5.27	6.48	5.84
6	8.16	6.05	5.30	4.26	6.06	7.04	6.63	6.60	5.92	5.85	4.80	4.59
7	3.29	4.57	2.71	2.40	4.44	5.38	5.05	3.71	3.29	3.12	3.13	3.23
8	2.49	2.60	2.43	2.27	2.53	3.58	4.11	4.12	3.74	3.97	4.50	4.77
9	7.49	6.93	6.57	6.02	6.47	6.73	6.43	8.07	7.89	7.73	8.72	6.95
10	6.77	5.47	4.41	3.47	4.69	5.45	5.49	4.72	4.17	3.95	3.68	2.86
11	9.77	8.05	7.95	6.13	6.69	6.40	6.69	7.19	6.82	6.82	7.51	6.29
Mean	5.76	5.13	4.49	4.10	5.01**	5.45**	5.85**	5.87**	5.75**	5.40**	5.50**	5.04**
SE	2.29	1.63	1.72	1.43	1.34	1.00	1.06	1.41	1.51	1.39	1.80	1.44
1.0 IU/ml (1–24)ACTH												
1	8.01	7.49	7.95	7.61	8.97	10.05	10.71	10.24	11.37	10.57	11.93	9.74
2	5.91	5.14	5.15	3.93	4.43	4.93	8.89	8.14	8.31	7.64	8.08	7.09
3	2.60	2.34	2.40	1.73	2.36	2.71	4.19	4.22	3.28	3.97	4.70	3.66
4	8.17	8.34	9.47	7.85	8.94	2.05	11.52	9.75	11.10	13.60	12.60	13.00
5	3.48	3.08	2.89	2.36	4.56	4.49	6.05	5.21	5.07	3.05	4.46	3.70
6	3.54	3.28	3.42	3.23	5.49	5.46	6.37	6.21	5.56	5.25	5.28	4.98
7	5.32	4.35	3.62	3.20	4.99	4.72	5.19	5.64	5.88	5.89	5.76	6.30
8	1.90	1.87	1.49	1.47	3.29	2.81	3.78	2.75	5.04	2.97	2.96	1.48
Mean	4.87	4.49	4.55	3.92	5.38*	5.90*	7.09*	6.52*	6.95*	6.62*	6.97*	6.26*
SE	2.38	2.37	2.80	2.49	2.41	3.36	2.94	2.64	2.98	3.80	3.57	3.72

persion. While such preparations possess several advantages in a number of applications (e.g. ACTH bioassay), physiological interpretations of results obtained using such cells is difficult since functions in these cells are significantly altered by the loss of surface receptors[17] and of the negatively charged extracellular matrix of the cells[18], and by the loss of cell-cell communication which has been implicated in the responsiveness of adrenocortical cells to stimulation[19, 20]. To obtain more physiological conditions, we used an alternative in vitro method which allows the measurement of basal corticosteroidogenesis and changes in steroid secretion after stimulation with ACTH up to 7 h[2–13]. The data presented in this study confirm and extend our previous work showing that corticosteroid secretion by adrenals of individual Mongolian gerbils superfused in vitro varied greatly from experiment to experiment[9–14] and that the response to (1–24)ACTH is relatively small[11] when compared to that of incubated tissue blocks[12]. Similar findings were reported on the rat adrenal gland superfused[2, 3, 5, 8] or incubated[21] in vitro. Saffran and co-workers[2, 3, 5, 8] suggested that sources of the observed day-to-day variations may be the age of the animals, time of day of the experiment, damage of tissue during preparation, length of preincubation or previous handling of the animals. The results described in this work demonstrate for the first time that (1–24)ACTH-stimulated secretion greatly depends on basal in vitro secretion of corticosteroids. While initial experiments with adrenal quarters suggested that the observed correlation may be explained by the differences in the number of cells which are in direct contact with medium or (1–24)ACTH during superfusion, experiments with slices show that this correlation did not depend on the mass of cells obtained in individual preparations and present in the superfusion flask, but rather depends on the individual secretion rates of tissue superfused in vitro (table 2). Interestingly,

Table 2. Corticosteroid secretion by Mongolian gerbil adrenal slices superfused in vitro: relationship between basal secretion and maximum output during (1–24)ACTH stimulation. After adrenal slices had been superfused with medium for 60 min, (1–24)ACTH (0.1 IU/ml) was added to the superfusion medium over 180 min and six 5-min samples were collected at 30-min intervals. The calculated regression line gave $y = 1.0x + 0.2$, where y = maximum secretion, x = basal (30 min) secretion; $r = 0.90$, $n = 27$, $p < 0.001$. * $p < 0.01$, ** $p < 0.001$ (Mann-Whitney U- test).

Experiment No.	Corticosteroid secretion (ng/adrenal pair/min) Length of superfusion (min) 30	60	90	120	150	180	210	240
a) Without (1–24)ACTH								
1	3.60	2.79	2.38	2.18	1.75	1.50	1.29	1.25
2	3.94	2.98	3.13	3.16	2.94	2.97	2.81	2.88
3	4.22	3.72	4.06	4.10	3.61	3.12	2.69	2.97
4	5.73	3.85	3.53	3.38	2.76	3.04	2.65	3.01
5	6.27	4.00	3.17	3.02	2.73	2.20	2.09	1.96
6	6.29	4.17	3.88	3.63	3.30	2.56	2.75	2.18
7	6.76	4.14	4.16	4.96	3.92	3.59	3.15	3.18
8	7.14	4.71	4.62	4.58	4.37	3.94	3.51	3.21
9	10.45	7.12	5.95	5.23	5.02	4.04	3.82	4.07
Mean	6.04	4.16	3.88	3.80	3.38	3.00	2.75	2.75
SE	2.09	1.25	1.02	1.00	0.98	0.82	0.75	0.83
b) With (1–24)ACTH								
1	4.81	3.36	3.95	4.24	4.75	3.30	2.18	2.10
2	5.06	4.31	4.79	4.49	5.09	4.08	3.43	2.58
3	5.38	4.73	6.95	8.04	7.70	5.69	4.02	3.78
4	5.45	3.76	5.09	5.61	5.43	4.28	3.51	2.88
5	5.54	4.85	6.91	7.00	8.24	6.41	6.31	5.44
6	5.56	4.08	6.36	6.08	6.59	5.23	5.07	3.84
7	5.80	4.06	5.24	5.12	4.91	3.86	3.48	2.89
8	5.82	4.37	5.99	6.32	6.18	5.48	4.58	3.44
9	6.12	5.64	6.82	7.94	8.94	6.16	6.23	4.99
10	6.40	4.79	6.09	6.80	6.45	5.16	4.28	3.30
11	6.36	4.74	6.38	7.62	7.88	4.39	3.33	3.03
12	6.55	4.73	4.82	4.82	4.80	4.88	3.55	3.60
13	6.66	5.20	7.04	7.60	7.38	5.81	4.33	3.88
14	6.84	5.27	9.17	8.12	8.05	5.02	4.15	3.06
15	7.18	5.66	6.48	6.25	6.39	3.82	2.97	2.17
16	7.32	4.20	5.38	6.12	5.60	4.84	5.05	4.38
17	7.80	5.32	9.96	9.50	9.51	6.42	5.21	4.31
18	8.09	7.72	10.85	10.94	11.65	9.69	7.63	7.80
19	8.39	4.12	5.65	5.82	5.44	4.40	4.60	3.24
20	8.66	6.14	7.15	6.97	7.02	6.14	4.82	4.05
21	8.93	6.52	7.96	8.65	8.43	7.44	5.87	5.57
22	8.96	4.33	7.01	6.74	6.40	5.08	4.56	4.30
23	9.44	7.15	11.62	11.62	12.06	8.89	7.63	6.43
24	9.84	6.12	10.70	11.08	10.07	8.57	8.31	7.09
25	11.65	6.68	12.57	13.22	14.69	10.36	6.86	5.96
26	13.50	7.55	11.16	11.55	11.16	9.40	8.14	6.64
27	18.54	11.77	19.36	16.00	17.09	16.04	16.41	15.06
Mean	7.80	5.45	7.79**	7.86**	8.07**	6.33**	5.43**	4.66*
SE	2.98	1.72	3.25	2.91	3.09	2.73	2.73	2.57

Experiment No.	Corticosteroid secretion (ng/adrenal pair/min) Length of superfusion (min) 30	60	90	120	150	180
1	4.78	2.73	4.00	6.26	6.50	6.70
2	5.42	2.37	6.72	9.02	9.30	10.49
3	5.83	2.33	6.38	7.97	7.89	8.51
4	6.84	5.75	16.97	16.63	17.47	18.07
5	7.35	3.89	6.10	10.05	11.79	11.86
6	7.73	3.91	11.08	14.49	17.18	18.06
7	7.99	4.87	15.41	18.53	18.64	16.32
8	8.10	4.11	12.67	20.06	20.49	18.90
9	9.09	6.95	12.09	14.57	12.86	12.06
10	10.60	7.68	16.38	22.82	25.67	27.55
11	10.83	4.60	11.14	21.31	38.83	37.49
12	11.45	7.16	18.15	22.29	22.06	20.10
Mean	8.00	4.70*	11.42*	15.33*	17.39*	17.18*
SE	2.98	1.72	4.76	5.87	8.98	8.62

Table 3. Corticosteroid secretion by adrenal quarters of the tree shrew *(Tupaia belangeri)* superfused in vitro: relationship between basal secretion and (1–24)ACTH- stimulated secretion. After adrenal quarters had been superfused with medium for 60 min, (1–24)ACTH (0.5 IU/ml) was added to the superfusion medium over 30 min and four 10-min samples were collected at 30-min intervals. The calculated regression line gave $y = 1.6x - 1.1$, where y = stimulated secretion (90 min), x = basal (30 min) secretion; $r = 0.70$, $n = 12$, $p < 0.01$. * $p < 0.01$ (Wilcoxon test).

these secretion rates could not be correlated to corticosteroid plasma concentrations ($r = 0.14$, $p > 0.05$) or adrenal weights ($r = 0.11$, $p > 0.05$). The importance of the findings presented here is further underlined by our very recent work which showed a positive correlation between basal and (1–24)ACTH-stimulated corticosteroid (table 3) and aldosterone/progesterone[22] secretion by the adrenal gland of the tree shrew *(Tupaia belangeri)* superfused in vitro. Similarly to the findings in the Mongolian gerbil, in vitro secretion of steroids by the tree shrew adrenal gland was not correlated either to corresponding plasma levels ($r = -0.41$, $p > 0.05$) or to adrenal weights ($r = 0.12$, $p > 0.05$)[22].

To summarize, superfusion of adrenocortical tissue from individual animals gives insights into aspects of adrenal function which cannot be investigated by static incubation of tissue blocks or isolated cells. The significant positive correlation between basal secretion and the responsiveness to (1–24)ACTH indicates that the fine adjustment of basal corticosteroidogenesis (also[11, 13, 23]) forms an important part of the regulatory mechanisms modulating (1–24)ACTH- stimulated corticosteroid secretion in vitro.

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Anomeric specificity of glucose-induced somatostatin secretion

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Summary. In isolated perfused rat pancreases, the α-anomer of D-glucose is more potent that β-D-glucose not solely in stimulating insulin release and suppressing glucagon output, but also in causing somatostatin secretion.
Key words. D-glucose anomers; endocrine pancreas; insulin; glucagon; somatostatin.

Several functional processes triggered by D-glucose (or D-mannose) display anomeric specificity. For instance, α-D-glucose is better able than β-D-glucose to evoke the taste of sweetness[1], to stimulate insulin release[2] and to inhibit glucagon secretion[3]. Likewise, in several cell types, including pancreatic islet cells[4–6], erythrocytes[7], adipocytes[8], hepatocytes[9] and brain cells[10], the metabolism of α-D-glucose differs from that of β-D-glucose. The latter difference persists in cells exposed to equilibrated D-glucose[8, 10]. The anomeric specificity of D-glucose metabolism is thought to result from adaptation to the anomeric environment, which is itself imposed by a thermodynamic constraint[11, 12]. The anomeric specificity of enzymes indeed underwent a phylogenetic evolution[13] and participates in the fine control of D-glucose catabolism[5, 12]. Moreover, the anomeric preference of functional processes is apparently perturbed in certain pathological situations[1, 14, 15]. These considerations justify, in our opinion, further efforts to assess the existence, mechanism and significance of such an anomeric specificity in distinct cell types. In this respect, the present report reveals that α-D-glucose stimulates more efficiently than β-D-glucose the secretion of somatostatin from the perfused rat pancreas.

Female Zucker rats (13–26 weeks old), fed ad libitum, were anesthetized with sodium barbital (42 mg/kg, i.p.), and the pancreas isolated from all adjacent organs and perfused through the celiac and superior mesenteric arteries[16]. The basal medium consisted of a Krebs-bicarbonate-buffer[17] containing L-leucine (10 mM), dextran T 70 (40 g/l) and bovine albumin (5 g/l). The anomers of D-glucose were dissolved in chilled saline and administered through a side-arm syringe maintained under ice. The flow rate was fixed at 1.6 ml/min. The methods used for the immunoassay of insulin, glucagon and somatostatin were previously described[17].

In each experiment, the α- and β-anomers of D-glucose (final concentration: 3.3 mM) were each administered twice for 15 min, followed by a 20-min period of glucose deprivation. The experiments were performed in 6 lean (192 ± 9 g b.wt) and 6 obese (284 ± 19 g) Zucker rats. In each type of rat, 3 animals received the D-glucose anomers in the sequence α1-β1-α2-β2 (α1 refers to the first administration of α-D-glucose, and α2 to its second administration) and the other 3 rats in the opposite order (β1-α1-β2-α2). No significant difference between lean and obese rats was found for the pancreas wet weight (0.64 ± 0.03 g), its somatostatin content (643 ± 39 ng/g), the basal somatostatin output (1.7 ± 0.6 pg/min) and the integrated somatostatin release during the entire experiment (238 ± 38 pg or, relative to the pancreas content, 0.06 ± 0.01%), the values quoted in parentheses representing pooled data (lean and obese; mean ± SEM; n = 11–12). The data for somatostatin release collected for one lean rat in