lysosomal alterations. Insulin probably acts by decreasing the fragility of the membrane of hydrolases-bearing particles and this effect is most likely due to a decreased formation of autophagic vacuoles. Such an inhibitory effect on the development of autophagy has been reported in adult rat liver: insulin inhibits autophagic-vacuoles formation in cultured hepatocytes[13,14] and in hepatocytes from perfused rat livers[15]. Hepatic autophagy in uncontrolled experimental diabetes may be corrected by insulin therapy[16].

Prenatal injection of glucagon does not lead to lysosomal alterations (tables 1 and 2), although glucagon under these conditions has a glycogenolytic effect (results not shown). It is suggested that the high fetal insulinemia[17] prevents the effect of exogenous glucagon. In adult rat liver, glucagon induces rapidly lysosomal alterations in animals fasted overnight, but not in fed animals[18]; possibly the decrease of plasma insulin level after short starvation period makes the hepatic lysosomes sensitive to the action of exogenous glucagon.

Our results indicate that a high level of plasma insulin during the perinatal period, whatever the glucagon level, prevents lysosomal alterations; and suggest that insulin lack by itself, which is the result of the fall of plasma insulin after birth, may largely contribute to the development of hepatic autophagy.

Table 2. Effect of insulin and glucagon on osmotic fragility of hepatic lysosomes during the perinatal period

	'Free' NAGase (per cent of total activity ± SEM)	'Free' acid phosphatase (per cent of total activity ± SEM)
Newborn, at delivery	30.2 ± 1.05 (8)	37.4 ± 1.47 (5)
Newborn, 2.5 h old		
Saline	46.2 ± 2.97 (5)	66.5 ± 2.01 (5)
Insulin	34.9 ± 1.71 (5)	46.5 ± 1.72 (6)
Fetus of 21.5 days		
Saline	27.9 ± 0.98 (6)	40.3 ± 1.51 (5)
Glucagon	29.2 ± 1.09 (6)	37.9 ± 2.12 (5)

Total and 'free' activities of NAGase and of acid phosphatase were assayed as given in materials and methods section. Numbers of experiments are given in parentheses.

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Corticosteroidogenesis by isolated human adrenal cells: Effect of serotonin and serotonin antagonists

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Summary. The direct effect of serotonin and antiserotonin agents on adrenal steroid biosynthesis was studied in isolated adrenal cells derived from patients with Cushing's syndrome. The results indicate that serotonin increases corticosterone production, while the serotonin antagonists cyproheptadine and methysergide depress adrenal steroid - particularly cortisol and aldosterone - biosynthesis.

Earlier studies utilizing in vitro systems have shown that serotonin, by direct action, enhances adrenal steroid synthesis in different species[2-4]. In isolated zona glomerulosa cells of the rat, serotonin in a concentration as low as 10^{-9} moles/l is capable of increasing significantly corticosterone and aldosterone production[2]. Based on these studies, it is now generally accepted that serotonin acts specifically on the zona glomerulosa and is one of the most potent stimulators of aldosterone biosynthesis in vitro[2-4]. It is therefore not surprising that serotonin antagonists decrease the aldosterone production of rat adrenal quarters and, when given concomitantly with serotonin, block the aldosterone stimulating effect of the latter[3]. The direct effect on steroid biosynthesis of serotonin and its antagonists has not so far been investigated in the hyperfunctioning human adrenal derived from patients with Cushing's syndrome due to ACTH overproduction.

Materials and methods. The study was made on isolated human adrenal cells obtained by surgery. The 1st patient had medullary carcinoma of the thyroid which caused ectopic ACTH syndrome, and was therefore subjected to bilateral adrenalectomy; histology of the adrenals revealed bilateral micronodular hyperplasia. The 2nd patient showed Cushing's syndrome of pituitary origin; histology of the removed adrenals again showed bilateral micronodular hyperplasia. Adrenal cells were isolated[5] using Collagenase Type I and DNA-ase I (Sigma Chemical Co. Ltd) and by mechanical dispersion[6]; the cells were resuspended in Krebs-Ringer bicarbonate buffer supplemented with glucose and albumin (KRBGA: pH 7.4; glucose: 0.2%; bovine serum albumin: 0.5%; K^+: 5.9 mmoles/l). Aliquots of 3×10^5 cell counts were incubated at 37 °C, in an atmosphere of 95% O_2 and 5% CO_2 for 120 min. Each substance tested was added to the medium at the beginning of incubation; the following compounds were used: $ACTH_{1-24}$ (Synacthen, CIBA Ltd, Basel), serotonin creatinine sulphate, and cyproheptadine hydrochloride (Reanal Chemical Works Ltd, Budapest), methysergide hydrogen maleinate (Sandoz Ltd, Basel). Steroid production was measured in 3 parallel cell cultures for each concentration of the

substances tested. Cortisol, corticosterone and aldosterone contents of the incubation media were established by radioimmunoassay[1,7-9] from 3 simultaneous measurements in each cell culture. The results obtained were analyzed statistically by using Student's t-test.

Results and discussion. Serotonin, in a concentration to be effective in rats adrenal cell systems in vitro, caused a significant increase of corticosterone production in the adrenal cell preparations of both patients with Cushing's syndrome, while cortisol and aldosterone synthesis was not affected.

The antiserotonin agents tested in the concentration used (10^{-5}–10^{-4} moles/l) failed to decrease corticosterone production significantly in the cell system derived from the patient with pituitary ACTH overproduction, whereas they were effective in blocking corticosterone synthesis by the adrenal cells of the patient with ectopic ACTH syndrome, but only when using the higher concentrations (10^{-4} moles/l). Cyproheptadine significantly reduced cortisol and aldosterone biosynthesis in both adrenal cell preparations even when used in the lower concentration (10^{-5} moles/l), while methysergide produced the same changes only in the higher concentration (10^{-4} moles/l). The results have been summarized in tables 1 and 2. In ectopic ACTH syndrome maximum steroid production was achieved with 100 pg/ml of ACTH, corticosterone increasing 4.5-fold, cortisol 9-fold, while aldosterone was not affected significantly. In pituitary ACTH overproduction, maximum steroidogenic effect was produced by 10 ng/ml of ACTH; the response was 4-fold for corticosterone, 10-fold for cortisol, and 2-fold for aldosterone.

The present results indicate that cyproheptadine, an agent known to benefit patients with Cushing's syndrome, relieves the symptoms not only by virtue of its central effect, i.e. by inhibiting ACTH secretion and thereby adrenal steroid production[10], but probably also by a direct inhibitory effect on adrenal corticosteroids. The peripheral site of action of cyproheptadine was suggested also by our earlier clinical study[11] in which we observed that sustained cyproheptadine treatment significantly blocked the rise in plasma cortisol and aldosterone levels that followed the administration of ACTH.

Remarkable that the aldosterone production was markedly depressed in the patient with pituitary ACTH overproduction. It should be stressed that, in both patients with Cushing's syndrome, the adrenals were being exposed to sustained ACTH stimulation as indicated by the raised peripheral plasma ACTH concentrations (250–500 pg/ml; normal range, 20–70 pg/ml), which might provide an explanation for the failure of serotonin to stimulate aldosterone production in the present cell systems. This situation mimics the effect of prolonged ACTH administration in normal subjects which results in the selective inhibition in the conversion of corticosterone to aldosterone, and refractoriness in aldosterone secretion to some aldosterone stimulating agents[12]. As known from animal experiments, the steroid production, and thus serotonin sensitivity of isolated adrenals in vitro, depends not only on previous sodium and potassium balance, or mineralocorticoid treatment, but also on sustained administration of exogenous ACTH[13]. It is also remarkable that the zona glomerulosa of rats treated with ACTH produces less aldosterone and deoxycorticosterone in vitro, and in these preparations aldosterone synthesis cannot be stimulated by serotonin[13]. Furthermore, aldosterone biosynthesis in rats is blocked in vitro by corticosteroids, e.g. cortisol, corticosterone, deoxycorticosterone[14], as also in patients with Cushing's syndrome where enhanced corticosteroid level may lead to intraadrenal inhibition of aldosterone production[15], also interfering with the effect of stimulating factors.

The present study on isolated human adrenal cells exposed to sustained stimulation by ACTH points to a different mechanism of corticosteroidogenesis affected by serotonin and its antagonists in vitro.

Table 1. Effect of serotonin on steroid biosynthesis of isolated human adrenocortical cells

	Ectopic ACTH production KRBGA (baseline): cortisol: 23.07 ± 2.39 corticosterone: 21.26 ± 0.41 aldosterone: 4.41 ± 0.22		Pituitary ACTH over production KRBGA (baseline): cortisol: 21.39 ± 2.0 corticosterone: 11.61 ± 1.5 aldosterone: 0.482 ± 0.07	
	10^{-5} moles/l	10^{-4} moles/l	10^{-5} moles/l	10^{-4} moles/l
Cortisol	25.7 ± 2.3	23.9 ± 0.55	22.74 ± 0.79	21.39 ± 1.43
Corticosterone	26.15 ± 1.6**		17.42 ± 1.53***	
Aldosterone	3.48 ± 0.22		0.574 ± 0.05	

*$p<0.05$; **$p<0.01$; ***$p<0.001$. Concentrations in the incubation medium for cortisol, corticosterone and aldosterone are expressed per cells 3×10^5, in ng/2 h (mean ± SD).

Table 2. Effect of cyproheptadine and methysergide on steroid biosynthesis of isolated human adrenocortical cells

		Ectopic ACTH production KRBGA (baseline): cortisol: 23.07 ± 2.39 corticosterone: 21.26 ± 0.41 aldosterone: 4.41 ± 0.22		Pituitary ACTH over production KRBGA (baseline): cortisol: 21.39 ± 2.0 corticosterone: 11.61 ± 1.5 aldosterone: 0.482 ± 0.07	
		10^{-5} moles/l	10^{-4} moles/l	10^{-5} moles/l	10^{-4} moles/l
Cortisol	Cyproheptadine	18.2 ± 2.5**	14.8 ± 2.4***	13.22 ± 1.54***	7.33 ± 0.92***
	Methysergide		17.3 ± 1.7**		10.54 ± 0.76***
Corticosterone	Cyproheptadine	18.54 ± 2.1	18.07 ± 0.46**	11.46 ± 1.02	8.34 ± 1.59
	Methysergide		17.92 ± 0.87**		10.88 ± 0.60
Aldosterone	Cyproheptadine	2.77 ± 0.41**	2.09 ± 0.31***	0.221 ± 0.06***	0.214 ± 0.07***
	Methysergide		3.35 ± 0.28*		0.264 ± 0.08**

*$p<0.05$: **$p<0.01$; ***$p<0.001$. Concentrations in the incubation medium for cortisol, corticosterone and aldosterone are expressed per cells 3×10^5, in ng/2 h (mean ± SD).

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DISPUTANDUM

Acupuncture points and cutaneous nerves

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Summary. In view of the number of workers who have confirmed the presence of cutaneous nerves beneath acupuncture points, a plea is made not to dismiss them too lightly or hastily at this point in the search for the mechanisms of acupuncture.

In a recent article in this journal Weidmann[1] surveyed the work in progress at the Shanghai Institute of Physiology, Division of Acupuncture. Quoting Chiang's work[2], Weidmann points out that skin nerves may be blocked by a local anaesthetic without loss of the analgesic effect of acupuncture applied to the same spot, whereas applying local anaesthetic to the muscle nerves makes needling ineffective.

As yet, persistent reports of a definite anatomical arrangement of structures at acupuncture points are lacking and indeed some of the findings are somewhat confusing if not contradictory. Recently Reichmanis and Becker[3] have reviewed some of the anatomical findings reported in the literature.

A number of workers have noted the close association of points with different neural structures, and Gunn et al.[4] proposed a classification of 70 acupuncture points according to their known neural structures, mentioning 3 types corresponding to: 1. the motor point of a muscle (35 points); 2. the focal meeting of superficial nerves in the sagittal plane (14 points); 3. points overlying superficial nerves or plexuses (21 points).

He is of course implying that at least some acupuncture points have some definite relationship with some superficial nerves.

Chiang[2] does not of course deny this and he injected local anaesthetic into a branch of the radial nerve which passes directly in the skin over Hoku (Co 4, L.I.4). Does this mean that this nerve has nothing to do with this acupuncture point but just happens to be passing here by chance?

Matsumoto and Lyu[5] have reported the correspondence between 33 acupuncture points and the usual points injected with local anaesthetic to produce regional/local nerve block.

Bossy et al.[6] performed dissection on 201 acupuncture points and reported macroscopic findings as follows: 58 points (29%) revealed a superficial cerebrospinal nerve, 74 points (37%) revealed a vasculo nervous pedicle, 69 points (34%) revealed a vascular element, mostly venous. They only dissected to a depth of 5 mm, and were thus only reporting 'supra-aponeurotic macroscopic elements'. However they claim that 132 points out of 201, i.e. 66%, revealed macroscopic superficial nerves. Is it just coincidence that these nerves are passing by acupuncture points?

A group of workers in Shanghai[7] have performed dissection on 324 points, not only superficially but also into deeper tissues as well. 8 cadavers, 49 upper limbs and 24 lower limbs were used in their studies although they do not state on how many of the parts each individual point was dissected. They reported finding macroscopic neural structures (nerves) beneath every point except one. Details of their findings are as follows:

superficial (cutaneous) nerves: 304 points
deep nerves: 170 points
both superficial and deep nerves: 149 points

The one remaining point together with 21 other important points were examined microscopically after staining and it was found that at each of these 22 points, from the skin down to deeper tissues (including muscle), in every layer there was an abundance of concentrated nerve bundles and fibres of varying diameters, and numerous dense nerve endings. Note especially that they found macroscopic evidence of cutaneous nerves at 304 of the 324 points examined i.e. 94%. Unfortunately fuller details have not yet been published to this author's knowledge.

My own studies including macroscopic dissection, microscopic sections and clinical examination have also shown that many acupuncture points have cutaneous nerves pass-