Effects of Estradiol and Cortisol on Neural Tissue in Culture[1]

The regulatory influence of hormones during the development of the central nervous system has been related to sensitivity of specific neural structures to hormones[2], to critical periods of development[3], and to hormonal specificity[4]. Although critical periods have been established, sensitivity of specific neural structures to hormones during development requires further investigation.

The present study investigates hormonal sensitivity of embryonic nervous tissues in culture. Explants of cervical spinal cord and cerebellum from 16-day-old chick embryos were used. The cervical spinal cord was removed from the vertebral column by means of the 'toothpaste tube' method of POMERAT[5], and was divided into 4 explants. The cerebellum was cut horizontally through the dorsal surface and 1 explant per cerebellum was removed. The explant contained 4–6 folia and extended from the molecular to the subcortical layer.

Explants were washed in EARLE's balanced salt solution[6] and oriented on a triangular stainless steel organ culture grid[7]. The ventral side of the spinal cord and the molecular surface of the cerebellum rested against the grid. Platforms with explant (1 explant per grid) were placed in organ culture dishes with a center well and an absorbent ring (Falcon Organ Culture Dish). The medium was added to the center well of the organ culture dish and did not reach the top of the platform. Humidity was maintained by saturating the absorbent ring with distilled water. The culture medium was EAGLE's basal medium[8] with EARLE's balanced salt solution with or without hormones. Estradiol dipropionate and cortisol (hydrocortisone free alcohol) were prepared in aqueous suspension, 1 mg/ml of EARLE's buffer solution to which Tween 80 had been added[9].

Explants were cultured at 35 °C for 24 h; the gas phase was 5% CO_2–95% O_2. Freshly prepared culture media were equilibrated with the gas phase prior to transfer of the explants. Organ culture dishes were stacked in the bottom portion of unsealed containers (plastic bread box, volume 4260 ml) and the gas was supplied at a constant flow to an inlet in the lid according to the method of LOSTROH[10].

The activity of acetylcholinesterase (AChE), the hydrolysing enzyme of acetylcholine (ACh), and total protein were measured. The AChE activity was measured colorimetrically by the method of ELLMAN, COURTNEY, ANDRES and FEATHERSTONE[11], and protein by the FOLIN-LOWRY reaction[12]. Data were obtained from 3 experiments. In each experiment the following groups were studied: (1) 6 non-cultured cerebellar and 4 spinal cord explants from 16-day-old chick embryos, (2) 6 cerebellar and 4 spinal cord explants cultured for 24 h on basal medium, (3) 6 cerebellar and 4 spinal cord explants cultured for 24 h on basal medium to which estradiol dipropionate, 50 µg/ml, was added, and (4) 6 cerebellar and 4 spinal cord explants cultured for 24 h on basal medium to which cortisol, 10 µg/ml, was added. To determine whether AChE activity and protein content in control and test explants differed significantly in their mean, the *t* test for non-paired data was applied[13].

In cerebellum and spinal cord explants cultured for 24 h on basal medium enzymatic activity and total protein decreased when compared to that of non-cultured tissues (Table). AChE activity and total protein remained at the basal level in spinal cord explants cultured on basal

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Acetylcholinesterase activity and protein content of cerebellum[a] and spinal cord[a] maintained as organ cultures

Culture medium[b]	Acetylcholinesterase activity µM AcTCh hydrolysed/min/g wet tissue		Protein content mg/g wet tissue	
	Cerebellum	Spinal cord	Cerebellum	Spinal cord
Non-cultured	5.33 ± 0.24[c]	22.77 ± 1.31	54 ± 1	51 ± 3
Basal	4.27 ± 0.21 (0.001–0.01)[d]	19.74 ± 1.22 (0.02–0.05)[d]	48 ± 1 (0.001–0.01)[d]	35 ± 4 (0.001–0.01)[d]
Estradiol	5.13 ± 0.26 (0.01–0.02)[e]	16.49 ± 2.01	47 ± 2	36 ± 4
Cortisol	5.04 ± 0.23 (0.01–0.02)[e]	18.58 ± 2.03	50 ± 2	39 ± 4

[a] Cerebellar and spinal cord explants were removed from 16-day-old chick embryos and maintained as organ cultures for 24 h. [b] The medium was EAGLE's basal medium with EARLE's salts. Hormones, when added, were in the following concentrations: estradiol dipropionate, 50 µg/ml, cortisol, 10 µg/ml. [c] Each value represents the mean ± SE of 18 cerebellar explants and 12 spinal cord explants. [d] Numbers in parentheses are *P* value for comparison to non-cultured control group. [e] Numbers in parentheses are *P* value for comparison to basal medium group.

medium containing hormones (Table). The spinal cord is morphologically, biochemically, and functionally mature in chicks by the 16th day of incubation[14]. It is suggested, therefore, that mature neural tissue is not influenced by these hormones.

AChE activity was significantly higher in cerebellar explants cultured on basal medium containing estradiol or cortisol than without hormone and did not differ from that in non-cultured 16-day-old cerebellar tissue (Table). The cerebellum is immature in 16-day-old chick embryos and AChE does not reach peak activity in chicks until 90 days after hatching[15]. Developing elements of the cerebellum may, therefore, be hormone-dependent for their growth and maintenance. Studies in this and other laboratories have given evidence that CNS growth may be significantly influenced by the presence of specific hormones[16]. Hormonal dependence for biochemical maintenance of neural explants cannot directly explain hormonal dependence of in vivo developing systems. Studies in vitro, however, may elucidate some of the underlying factors involved in neural hormonal sensitivity during development[17].

Résumé. L'activité acétylcholinestérasique et la teneur globale en protéines de fragments de cervelet et de moelle d'embryon de poulet de 16 jours diminue lorsqu'ils sont maintenus en culture organotypique dans le milieu standard de EAGLE. Après addition de cortisol ou d'oestradiol au milieu, les caractéristiques des tissus ne sont pas alterées par la mise en culture.

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Enhanced Hypoglycaemic Effect of Exogenous Insulin Associated with an Increased Response of Adipose Tissue and a Diminished Response of the Diaphragm in 'Meal Fed' Rats

Periodic hyperphagia evoked by infrequent feeding (e.g. training rats to consume their daily ration within 2 h or force feeding them twice a day by stomach tube) leads to enhanced lipogenesis[1-5] and may result in an increased amount of body fat – 'obesity without overweight'[6-9]. As MAYER[10] has pointed out, obesities of varying etiology differ greatly in many features, including the response to hormone administration. Thus the insulin sensitivity or goldthioglucose-treated obese mice was found to be normal, whereas mice with the hereditary obese hyperglycaemic syndrome are extremely resistant even to large doses of insulin[11,12]. This work was undertaken to assess the influence of feeding periodicity on the sensitivity of rats to exogenous insulin.

Female Wistar rats weighing 150–250 g and fed a standard laboratory diet[13] were used. The animals had either free access to food (controls) or were allowed to eat only for 2 h each day (from 07.00–09.00); these are referred to as 'meal fed' rats. All animals had water ad libitum. The hypoglycaemic effect of insulin was estimated after at least 5 weeks of experimental feeding. Furthermore, the glycogen content and in vitro $^{14}CO_2$ production by parametrial adipose tissue and diaphragm and incorporation of ^{14}C-U-glucose into total lipids of adipose tissue was measured in order to throw some light on the

Table I. The effect of crystalline insulin on blood glucose levels of control and 'meal fed' rats

Experimental group	Doses of insulin U/kg body weight	Blood glucose initial level mg/100 ml	Blood glucose changes after insulin injection (% of initial level)			
			30 [a]	60 [a]	120 [a]	240 [a]
Control (5)	0.1	82.0 ± 3.9	101.4 ± 6.9 [b]	88.2 ± 5.6	79.0 ± 3.8	88.3 ± 5.6
'Meal fed' (6)		72.0 ± 4.6	92.1 ± 8.8	60.2 ± 6.9 [d]	74.9 ± 7.2	81.1 ± 6.2
Control (10)	0.2	91.3 ± 7.36	76.3 ± 6.5	55.4 ± 5.6	47.1 ± 3.7	70.9 ± 3.8
'Meal fed' (11)		83.7 ± 1.45	55.8 ± 7.7 [c]	38.0 ± 2.4 [e]	41.7 ± 3.5	64.5 ± 3.1
Control (5)	0.4	85.6 ± 3.13	55.9 ± 3.9	56.0 ± 3.6	57.9 ± 4.1	81.9 ± 4.8
'Meal fed' (5)		92.6 ± 2.50	48.4 ± 4.6	43.0 ± 1.9 [d]	47.4 ± 3.9	78.6 ± 3.1

The figures in parentheses indicate the number of rats in each group. [a] Min after insulin injection. [b] Mean values ± standard error of mean. Symbols for statistical significance of differences between compared group averages: [c] ($P < 0.05$); [d] ($P < 0.02$); [e] ($P < 0.01$).