Effects of altered thyroid state on the inhibition produced by locus coeruleus in Purkinje cells in the rat

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Summary. The number of cerebellar Purkinje cells inhibited after locus coeruleus stimulation was found to be greater in hyperthyroid rats than in control healthy animals; these in turn showed a higher percentage of inhibited cells than hypothyroid rats. It is concluded that thyroid hormone is capable of modulating synaptic activity in the LC-PC pathway.

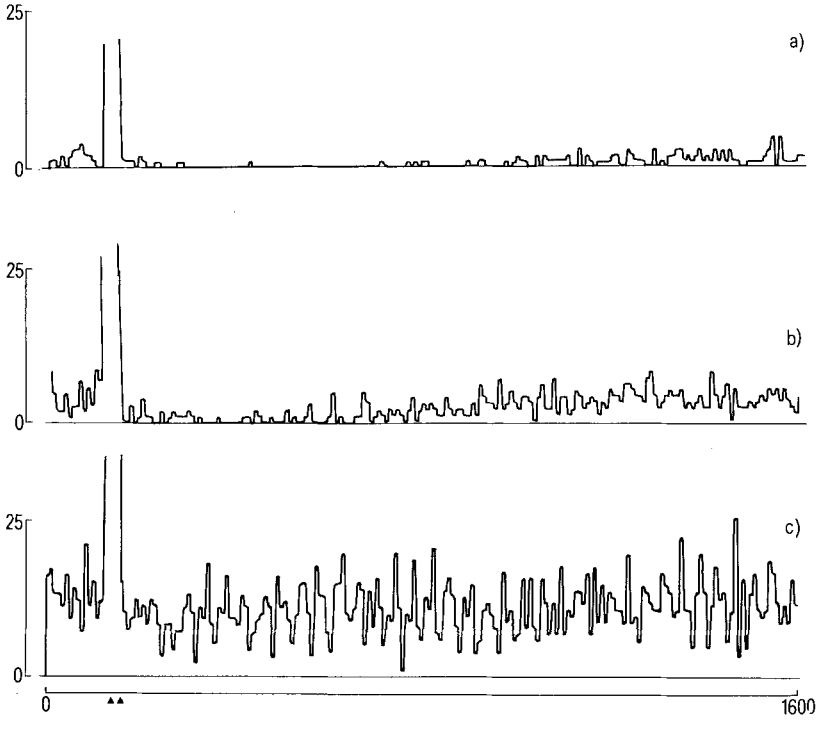
Previous work from this laboratory has shown that brain stem cell responses to iontophoretically applied noradrenaline (NA), vary according to the thyroid state of the animal[1]. As this finding was reported to occur in unidentified cells, it was thought necessary to investigate whether similar changes take place in an identified noradrenergic tract within the central nervous system (CNS). The locus coeruleus (LC) is such a tract, the noradrenergic character of which is well established[2], and its projection terminal fields are well known[3]. One place is the cerebellum where LC fibres terminate onto Purkinje cells (PC). It was decided to investigate the possibility of changes induced in NA-mediated LC effects on PC, in animals with altered thyroid state, as assessed by the effect of stimulation of LC on unit activity of PC.

Materials and methods. Sprague-Dawley rats of either sex, weighing 200–300 g, were divided into 3 groups: one served as control, one group was made hyperthyroid and the other one hypothyroid. Hormonal and chemical manipulations as well as controls of thyroid state were essentially similar to those used in our previous work[1]. After a suitable period, recordings were made. A stimulating electrode was stereotaxically lowered into the LC at coordinates AP9; L3; V6[4], and recordings were made using glass micropipettes filled with Pontamine sky blue dissolved in 0.5 M sodium acetate. The LC was stimulated with trains of stimuli (0.1 msec duration, 20 V) train duration was 30 msec at 250 Hz, repeated every 2 sec. Recordings were made in folia IV, V and VI of the ipsilateral cerebellar vermis. Post-stimulus time histograms (PSTH) were made with a computer by adding 100 responses (8–10 msec/bin, 200 bins). All recording and stimulating sites were histologically checked, and only results in which the stimulating electrode was within the LC were considered.

Results and discussion. A total of 68 PC were studied. Responses to LC stimulation were similar to those already described[5], in short, 2 types of responses were encountered: a) long duration inhibition (longer than 400 msec), b) short duration inhibition (less than 70 msec). When the number of cells inhibited in each group was analyzed, it was found that the highest percentage belonged to the hyperthyroid group, and the lowest to the hypothyroid group, control animals being midway between the two. A χ^2 analysis showed statistically significant differences - between the hypo- and hyperthyroid groups in relation to the control group (hyper vs control $p < 0.05$; hypo vs control $p < 0.01$). These results are summarized in the table. Although no detailed analysis was performed, the impression was gained that the extent of the inhibitory period was also changed, as illustrated in the figure. It is shown that hyperthyroid animals have longer periods of inhibition than controls, and in turn control inhibitions are longer than in hypothyroid animals.

	Number of cells	Inhibited	No effect	% of inhibited cells
Control	25	21	4	84
Hyperthyroid	21	19	2	90.4
Hypothyroid	22	7	15	31.8

Percentage of cells inhibited after LC stimulation in each of the experimental groups. χ^2-analysis (Yates correction): control vs hyperthyroid differ significantly at $p < 0.05$; control vs hypothyroid at $p < 0.01$.



PSTHs of 3 cerebellar Purkinje cells after stimulation of locus coeruleus nucleus. *a* Hyperthyroid; duration of inhibition = 830 msec. *b* Control; duration of inhibition = 700 msec. *c* Hypothyroid; duration of inhibition = 300 msec. Abscissa: spikes/address. Ordinate: time in msec. Solid triangles mark stimulus artefact. 100 responses; 8 msec/bin; 200 bins.

The present results show that the effects of a specific NA-releasing tract can be modulated by the amount of circulating thyroid hormone, and reinforces the contention that this is one of the effects of this hormone in the CNS. At present it is not possible to decide if this effect is presynaptic, as has been proposed for the actions of TRH[6], or postsynaptic. However, iontophoretic application of NA onto PC (to be reported elsewhere), gave similar results to those described for brain stem cells[1], which suggests a probable postsynaptic site of action.

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Microsphere measurement of myocardial capillary bed in hypoxic rats

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Summary. Rats exposed to hypobaric hypoxia for 24 h showed a significant increase in the number of perfused capillaries and in the number of radioactive microspheres trapped in the coronary circulation.

It has been well established that the heart undergoes hypertrophy in mammals subjected to chronic hypoxia. In an attempt to clarify cellular events that occur in the heart during the early stages of exposure, previous work in our laboratory has shown that young adult rats exposed to a moderately severe level of hypobaric hypoxia (380 torr) during a 24-h sojourn in a decompression chamber exhibited a statistically significant increase in heart weight when compared with rats maintained at ambient pressure (725 torr)[1,2]. Ventricular tissue from hypoxia exposed rats weighed more than ventricular tissue from control animals on both an absolute and a relative (mg heart/100 g b.wt) basis. Body weight, on the other hand, decreased precipitously during 24 h of hypoxia exposure (mean: −33 g/rat) while control animals gained about 6 g per rat during the same time period. Cytophotometric measurements of RNA in cardiac myocytes showed slightly lower values for azure B-stained RNA in the cytoplasm of heart muscle cells from 1-day hypoxia exposed rats when compared with corresponding controls, an indication that augmented protein synthesis is probably not a factor in the observed increase in heart weight during the 1st day of hypoxia exposure[2]. Histological studies also revealed a significant increase in the number of capillaries per unit area of ventricular myocardium in heart sections from hypoxic rats[3].

Summary of experimental data

Parameter	24-h Hypoxia exposed (N = 12)	Control (N = 14)
Body weight		
at autopsy	295 ± 4 g*	323 ± 12 g
initial	328 ± 5 g	319 ± 13 g
change	−33 ± 2 g	+4 ± 2 g
Heart weight		
actual	807 ± 17 mg	805 ± 24 mg
mg heart/100 g b.wt	273 ± 7 mg	250 ± 6 mg
Capillaries/mm^2	1380 ± 47	902 ± 35
^{153}Gd microspheres		
total cpm injected	27×10^4	39×10^4
cpm/heart ventricles	2.5×10^4	2.1×10^4
cpm/mg heart	33 ± 24 (NS)	25 ± 15
% recovery	9 ± 5 (NS)	5 ± 2

* All values are expressed as mean ± SEM. ** Value is percentage of injected counts recovered in the heart ventricles.

The present study was designed to utilize the radioactive microsphere technique in an attempt to obtain a more accurate assessment of the extent of coronary vascularization resulting from exposure to hypobaric hypoxia. The validity of the microsphere technique for obtaining data of this type has been well established[4].

Methods. Male Wistar albino rats were maintained continuously at 380 torr in a ventilated decompression chamber; controls were maintained at an ambient pressure of 725 torr. At the end of 24 h rats were anesthetized with pentobarbital (35 mg/kg, i.p.) and a polyethylene catheter was inserted through the right common carotid artery and into the left ventricle, using the procedure reported by Sasaki and Wagner[5]. Approximately 50,000 microspheres, 15.3 μm in diameter, labeled with ^{153}Gd (New England Nuclear) and suspended in 0.05 ml of 10% dextran plus 0.01% Tween 80, were injected into the left ventricle, followed immediately by 0.3–0.4 ml of heparinized physiological saline. Total injection time was 1 min. Microsphere suspension was contained in a silastic tube (7.5 cm long, 1 mm inner diameter) which was attached to end of implanted catheter. Total amount of radioactivity injected was calculated from the difference between initial amount and that remaining in silastic tube after injection and saline flush. Ventricles, right lung, both kidneys were excised, fixed in 10% neutral buffered formalin for 24 h, transferred to fresh formalin and gamma radioactivity was counted using a Beckman Biogamma II. Ventricles were processed by the paraffin method, 8-μm sections stained with hematoxylin and eosin were used for capillary counts. An ocular reticle engraved with 1-mm^2 was used to delimit areas to be counted; capillaries were counted only in regions where muscle fibres were cut in cross section.

Results and discussion. Table 1 summarizes experimental data. Body weight changes were almost identical to those found previously but, unlike earlier studies[1,2], heart weights showed little difference between hypoxia-exposed and control animals. Relative heart weight (mg heart/100 g b.wt) was only 9% greater in hypoxia-exposed rats. Capillary counts in the ventricular myocardium showed 53% more perfused capillaries in hypoxic hearts (i.e., a change from 902 to 1380 capillaries/mm^2). Data on the number of microspheres trapped in the myocardial capillaries suggest an enhancement of coronary circulation in hypoxia exposed rats. This is supported by the finding that total counts in ventricular tissue and the percentage of injected radioac-