facts[8] and prevent the development of tachiphylaxis. In addition, in some experiments the same electrode was used to record from control and from experimental animals, in an effort to compensate for changes due to different transport numbers in different pipettes. All recordings were made from 120 spontaneously active, unidentified brainstem neurones.

Results and discussion. NA responses were qualitatively similar to those previously described[9], but excitation was found more frequently than inhibition, so the former response was used in this study. Once a responsive unit was found, NA was applied at several currents, and a similar schedule was used with all the drugs tested. Threshold and maximal responses were not investigated. The averaged firing frequency measured in the minute preceding a 30 sec application, was compared to the maximum frequency attained after the application, and this was expressed as a percentage increase above the control frequency. Dose-response curves were constructed by drawing regression lines, using all data points obtained for a particular drug, from all animals in a group.

The figure shows the response to NA. The dose-response lines corresponding to experimental animals are displaced significantly in relation to control animals, suggesting decreased responsiveness in hypothyroid animals and increased responsiveness in hyperthyroid rats. A similar analysis of acetylcholine and glycine responses did not show any significant change (data not shown).

The present results are similar, at the single unit level, to those already quoted[2-6] which have used different paradigms of CA action, and suggest that this could be a general action of thyroid hormone in adrenergic systems. It is premature to speculate on the exact mechanism of action by which this effect is brought about, but experiments (to be reported elsewhere) show that acute administration of thyroid hormone by microiontophoresis does not produce the same effect, so it would appear that chronic administration is necessary to produce the change described. It is interesting to note that thyroid hormone can be taken by nerve terminals[10], and Dratman[11] has proposed that thyroid hormone could also act as an amino acid analog of tyrosine, whose uptake and subsequent metabolism by nerve tissue could lead to the formation of false transmitters, and these in turn could produce post-synaptic supersensitivity if released in sufficient amounts. Alternatively a certain level of thyroid hormone may be necessary for adequate synaptic function, levels above or below leading to hypersensitivity or hyposensitivity respectively.

The results reported could also have some bearing upon the explanation of the enhancement of the antidepressive action of imipramine produced by thyroid hormone[12], as CA have been involved in the pathogenesis of depression[13].

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PRO EXPERIMENTIS

Influence of hydrocortisone on cytopathic effect of Newcastle disease virus and stability to freezing of vescicular stomatitis virus[1]

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Summary. The presence of hydrocortisone in virus-infected cell cultures leads to enhancement of the syncytia forming ability of Newcastle disease virus and to production of vescicular stomatitis virus particles which loose their infectivity upon storage below 0 °C.

In the course of investigations on the effects of steroid hormones on virus growth and cytopathology, it has been observed that hydrocortisone enhances the ability of Newcastle disease virus to form syncytia and leads to production of vescicular stomatitis virus particles which are inactivated when stored below 0 °C. Preliminary data from these researches are referred to herein.

Materials and methods. Hydrocortisone hemisuccinate was furnished by Sigma. Virus strains were Newcastle disease virus (NDV) and vescicular stomatitis virus (VSV), both used in previous researches[2]. Experiments were carried out on aneuploid HEp2 cells (ATCC, Rockville), grown in Eagle's minimum essential medium (MEM) brought at pH 7.3, and supplemented with 7% calf serum. 16-h-old cell monolayers (10^6 cells/sample) were infected with 10 virus plaque forming units (PFU) per cell for 1 h at 20 °C, washed 3 times in Hanks' balanced saline solution (BSS, pH 7.3) and incubated at 37 °C in Eagle's MEM (pH 7.3) containing 2% calf serum. Hydrocortisone was added to the infected cultures in decreasing concentrations, starting from the maximum non-cytotoxic dose (MNCTD) established as previously described[3]. Virus-induced cytopathology was evaluated by microscope examination of cell monolayers stained by Giemsa. To quantitize infectious virus yield, whole culture samples were sonicated (40 Hz for 1 min) and stored at either above or below 0 °C prior to being cleared of cell debris at 3000 rpm for 5 min. Appropriate dilutions of supernatants thus obtained were tested for the content in PFU by the agar method[4]. An error of about 23% was found for both viruses.

Results. Table 1 shows the results obtained from tests in which hydrocortisone was added to cell cultures soon after

Table 1. Effect of hydrocortisone on growth and cytopathology of NDV and VSV

Hydrocortisone hemisuccinate in the medium (μg/ml)	VSV (after 20 h at 37 °C) PFU	CNE	NDV (after 20 h at 37 °C) PFU	CNE	SYNC
-	1.6×10^7	+ + +	9.6×10^6	+ + +	±
200	3.5×10^4	+ + +	7.6×10^6	±	+ + +
50	6.8×10^5	+ + +	8.5×10^6	±	+ + +
12.5	2.1×10^7	+ + +	1.1×10^7	+ +	+ +

PFU, plaque forming units; CNE, cytonecrotic effect; SYNC, fusion of cells into syncytia. + + +, Effect involving all cells; ±, effect involving very few cells.

Table 2. Effect of storage temperature on infectivity of VSV particles in the presence of hydrocortisone

Growth medium	Sonication after growth	6-h storage in MEM 2% serum	Infectivity (in PFU)*
MEM 2% serum	+	-	3.5×10^7 (1.1×10^7-5.6×10^7)
	+	+30 °C	2.9×10^7 (1.6×10^7-3.9×10^7)
	+	+20 °C	3.1×10^7 (9.8×10^6-5.2×10^7)
	+	+ 4 °C	1.8×10^7 (8.6×10^6-2.5×10^7)
	+	− 4 °C	2.5×10^7 (1.4×10^7-3.8×10^7)
	+	−30 °C	2.1×10^7 (1.6×10^7-5.1×10^7)
MEM 2% serum	+	-	2.1×10^7 (1.5×10^7-3.8×10^7)
+ Hydrocortisone 150 μg/ml	+	+30 °C	1.8×10^7 (1.1×10^7-2.4×10^7)
	+	+20 °C	1.6×10^7 (8.4×10^6-3.0×10^7)
	+	+ 4 °C	2.3×10^7 (1.2×10^7-3.6×10^7)
	+	− 4 °C	4.0×10^4 (2.0×10^4-8.0×10^4)
	+	−30 °C	3.0×10^4 (6.0×10^3-5.0×10^4)
MEM 2% serum	+	+20 °C + Hydrocortisone: 300 μg/ml	1.6×10^7 (8.4×10^6-2.2×10^7)
	+	+ 4 °C + Hydrocortisone: 300 μg/ml	2.1×10^7 (1.2×10^7-4.1×10^7)
	+	− 4 °C + Hydrocortisone: 300 μg/ml	1.1×10^7 (9.5×10^6-2.3×10^7)
	+	−20 °C + Hydrocortisone: 300 μg/ml	1.3×10^7 (9.5×10^6-2.5×10^7)

* Each value is the mean of 4 experiments. Extreme values are given in parenthesis.

infection, and production of infectious progeny was determined starting from whole culture samples, sonicated and frozen at −30 °C for 5 h. The data demonstrated that hydrocortisone markedly influences cytopathic effect of NDV by reducing cytonecrosis and increasing fusion of cells into syncytia, typical of this and other paramyxoviruses[5]. In addition, a strong decrease of VSV yield is observed in hydrocortisone-treated cell cultures. Since the last finding emerged rather unexpected, and nothing similar appeared in the literature, the possibility was envisaged that, rather than preventing growth of VSV, hydrocortisone might produce organization of labile virus particles sensitive to certain extraction procedures (freezing, in that case) which precede titration of virus progeny. Therefore, in successive tests, carried out as before, several extraction methods were used, and titrations repeated. Data obtained in these experiments (table 2) can be summarized as follows: in the presence of hydrocortisone, VSV particles are produced which maintain the same infectivity as that of untreated controls when extracted from cell cultures by sonication and kept in liquid environment from 0 °C up to 20 °C, but lose 3 logs of infectivity when frozen (−4 °C or −30 °C). Hydrocortisone has no such effect if added to virus suspension directly, before freezing.

Experiments are in progress to establish the mechanism by which hydrocortisone influences the cytopathic effect of NDV and labilizes VSV particles. Besides any other considerations, however, the data we obtained show that freezing below 0 °C, widely used both to free virus particles from cells and to maintain virus infectivity, may actually produce loss of infectivity at least under certain conditions.

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