THE IN VITRO EFFECT OF PARATHYROID EXTRACT ON THE AMOUNT OF NADP IN EMBRYONIC MOUSE CALVARIA.

by

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Introduction

This study is a continuation of the investigations of Gaillard, Hekkelman, de Voogd van der Straaten and Herrman-Erlee (1964) into the mode of action of parathyroid hormone on bone metabolism. From enzymological studies of Hekkelman (1961, 1963, 1965) concerning the extractability of isocitric dehydrogenase from homogenized bone tissue and the effect of NADP and NADPH thereupon, the hypothesis arose that the quantity of NADP and/or NADPH available in bone cells decreases under the influence of parathyroid hormone. In good confirmation with this hypothesis, de Voogd van der Straaten (1963, 1965), using tissue culture methods, observed the hormone action to be antagonized by adding NAD or NADP. However, direct proof was lacking, because of difficulties turning up in the estimation of nicotinamide nucleotides in diaphyseal bone preparations (Hekkelman, 1965).

In view of the important role which NADP most probably plays in the action of parathyroid hormone on bone metabolism, we intended to get direct information about the actual level of this coenzyme in bone tissue.

Using Lowry's very sensitive method of enzymatic cycling (Lowry, 1961) for the determination of pyridine nucleotides, it is possible to estimate both oxidized and reduced coenzymes in micro-quantities of tissue. Taking into account the above mentioned considerations we started our investigations with NADP.

Materials and methods

Using a roller tube technique (Gaillard, 1960, 1961[a,b].) control and experimental calvarium halves from 18-day-old mouse embryos were cultivated for 4, 6, 10 or 16 hours in a standard medium or in a medium also containing 1 IU parathyroid extract (Lilly). Care was taken to add the same amount of phenol to the control cultures as was given with the parathyroid extract to the experimental cultures. For each experiment 12 embryos belonging to two different litters (6 embryos per litter) were used. From each whole calvarium the left halve was cultivated in the control medium and the corresponding right halve in the experimental medium. At the end of each culture period the calvarium halves were washed in cooled (about 4°C.) Hank's solution and quickly frozen in liquid air. After lyophilization (0.05 mm Hg) at -25°C. during 16 hours the calvarium halves were pooled in groups of two, weighed (0.5 - 1.2 mg) and immediately thereafter homogenized at 0°C. in 0.5 ml 0.01M H_2SO_4 - 0.1M Na_2SO_4, pH 2.3, with subsequent heating for 30 minutes at 60°C.

According to Lowry (1961), "this treatment reduces DPN-ase and TPN-ase to a point at which it will ordinarily not be disturbing, although some activity may persist". Insoluble material was not removed and NADP was measured by enzymatic cycling. From each homogenate two samples (10 µl) were analyzed on each day of assay. All values were recorded as 10^{-6} moles per kg. of dry weight.

TABLE I

Levels [x] and procentual variations [xx] of NADP values in calvarium explants

Incubation period in hours	Number of determinations	Control explants (a)	PTE (1 IU/ml) treated explants (b)	Procentual decrease $(\frac{a-b}{a} \cdot 100)$	P values
4	5	68.7 ± 5.4	60.7 ± 6.4	3.6 ± 8.1	not sign.
6	5	45.5 ± 3.7	36.9 ± 2.0	18.5 ± 3.2	< 0.02
10	9	71.4 ± 7.3	49.3 ± 4.1	29.5 ± 3.5	< 0.001
16	5	65.0 ± 5.1	17.7 ± 1.8	72.7 ± 2.1	< 0.001

x) All values are expressed as μM/kg dry weight, together with the standard error of the mean.

xx) Results are given as the mean percentage ± standard error of the corresponding control values.

Results

Table I summarizes the values for NADP present in 18-day-old mouse calvaria after cultivation in a standard medium or in a medium to which PTE was added (1 IU per ml). Considering the values for the control and experimental explants after different periods of incubation (vertical columns) it is clear that they vary rather considerably. This seems to be true also for NADP estimations in other tissues. In developing rat liver Burch and von Dippe (1964) found for instance about 42 % differences in total NADP between livers obtained from 18- or 19-day-old rat embryos. Therefore it might be possible that also in calvarian bone variations in the actual age of the embryos occur and consequently might be responsible for the differences mentioned in the Table. Further investigations in this respect seems necessary.

Comparing however the values of the controls with those obtained from their paired experimental explants (horizontal lines) it is clear that the latter ones are consistently lower. Calculating the procentual differences (see before last column) and applying Student's t-test for paired observation the PTE dependent decrease indeed appears to be highly significant for incubation periods of 6, 10 and 16 hours but not for the 4-hour period. Moreover it is apparent that the values of the "procentual decrease" indeed increase as the time of incubation increases.

In conclusion it may therefore be stated that in explanted embryonic mouse calvaria added PTE does indeed cause a decrease of NADP thereby confirming Hekkelman's hypothesis as it is mentioned in the introduction of this paper.

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