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Action of TSH on nuclear ADP-ribosylation in dog thyroid slices

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Summary. Treatment of dog thyroid slices with thyrotropin (TSH) results in an increase in ADP-ribosylation in nuclei isolated thereafter. This increase is time-dependent and is observed with concentrations of TSH eliciting physiological responses. The technique described here does not involve permeabilization of cell membranes, thereby avoiding artefacts which could arise from hypotonic shock. Cyclic AMP mimicked the stimulatory action of TSH.
Key words. ADP-ribosylation; thyroid; thyrotropin; cyclic AMP.

Post-translational modification of proteins include a varitey of reactions, such as methylation of arginine residues[1], phosphorylation[10] and ADP-ribosylation[6,12]. In this last reaction, acceptor proteins are mono- or poly-ADP-ribosylated from NAD and this biochemical event has been proposed to be linked to DNA replication, cell proliferation and refractoriness to hormones. An enzyme that catalyzes ADP-ribosylation has been found in different subcellular fractions both in eukaryotes and in prokaryotes[6,12]. ADP-ribosylation has been shown to be under hormonal control, for example variations in the amounts of monomeric and polymeric ADP-ribose residues have been demonstrated in the mouse kidney after castration and testosterone treatment[5], and in the mouse liver after thyroidectomy and thyroxine treatment[9]. As far as the thyroid is concerned, TSH has been reported to increase total cell ADP-ribosylation in permeabilized cells[4] and in membranes of bovine and rat tissue[2,14]. The aim of the present study was to investigate the action of TSH on ADP-ribosylation in purified dog thyroid nuclei.

Materials and methods. Dogs were pretreated with thyroid powder for one day, as already described[13], and the thyroid glands were obtained by surgery under pentobarbital anesthesia. Slices were obtained with a Stadie-Riggs microtome (A. Thomas) and preincubated under 95% O_2/5% CO_2 for 30 min at 37 °C in Krebs-Ringer bicarbonate buffer (KRB) pH 7.4 containing 8 mM glucose and 1 mg/ml of bovine serum albumin. At the end of this period the slices were transfered to flasks containing fresh buffer, with or without TSH, and incubated for different lenghts of time. Approximately 1 g of slices was incubated in 7–10 ml KRB. The slices were then homogenized in 0.32 M sucrose, 30 mM Tris-HCl pH 7.8, 1 mM $MgCl_2$, 1 mM dithiothreitol (DTT) with a Potter Elvehjem tissue homogenizer with a teflon pestle, at 4 °C. The homogenate was filtered through gauze and centrifuged at 800 × g for 10 min. The resulting pellet was resuspended in 3 ml 2.1 M sucrose in the same buffer, and centrifuged at 50,000 rpm for 60 min in a SW_{56} rotor[7] to provide a nuclear pellet. Each pellet was resuspended in 200 µl of 30 mM Tris buffer pH 7.8 containing 25% (v/v) glycerol, 1 mM DTT and 1 mM $MgCl_2$. To this suspension 550 µl of 30 mM Tris-HCl buffer pH 7.8, 80 mM KCl, 10 mM $MgCl_2$, 1 mM DTT, 1 mM ATP and 0.1 mM NAD were added. Aliquots of 150 µl of the resuspended nuclei were utilized for the assay. DNA content was determined fluorometrically with ethidium bromide[11], after RNAse treatment.

ADP-ribosylation was measured following the method of Kostka and Schweiger[8], by incubating the purified nuclei in triplicate with a tracer amount (usually around 100,000 cpm) of ^{3}H-NAD (2,8 ^{3}H-NAD, New England Nuclear; 3.4 Ci/mmole sp. act.). After 6 min at 25 °C the reaction was stopped by the addition of 0.5 ml of cold 20% (w/v) TCA. The precipitates were filtered and washed with 10% TCA on GF/C Whatman glass fiber filters on a Millipore 1225 apparatus. The filters were dried,

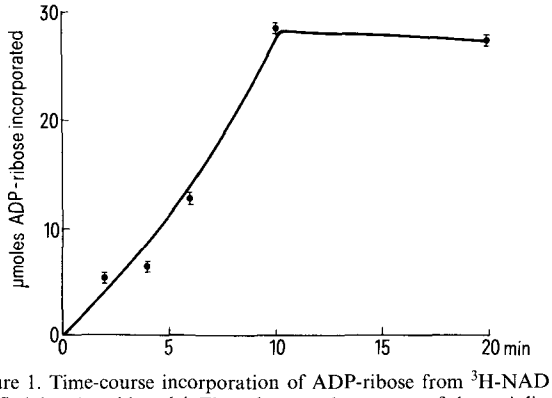

Figure 1. Time-course incorporation of ADP-ribose from ^{3}H-NAD by purified dog thyroid nuclei. The values are the average of close triplicates from a typical experiment. Each incubation tube contained purified nuclei corresponding to 65 mg of fresh thyroid.

Concentration-response relation of the action of TSH

Treatment	pmoles ADP-ribose/μg DNA	p <
Control	94 ± 22	
TSH 0.1 mU/ml	79 ± 9	NS.
TSH 0.5 mU/ml	271 ± 11	0.01

Thyroid slices were obtained from dogs pretreated with thyroid powder and incubated for 1 h with the indicated amounts of TSH. Nuclei were purified and ADP-ribosylation was measured (see 'materials and methods'). Each value is the average of triplicate determinations ± SD from a representative experiment.

digested with 0.5 ml Soluene (Packard) for 1 h at 50 °C, mixed with 7 ml Insta-Fluor (Packard) and the radioactivity counted by liquid scintillation spectrophotometry. Zero time blanks were substracted from each group.

In some studies purified nuclei were incubated under the same conditions and at the end of the labeling period they were rapidly centrifuged at 800 × g for 10. The resulting pellet was resuspended in the assay buffer described above and 50 μl aliquots were incubated, in triplicate, for varying times from 2 to 20 min. The incubations were terminated by the addition of 0.5 ml of cold TCA, the samples were filtered and their radioactivity counted, as described above. TSH, as Thytropar, was obtained from the Armour Pharmaceutical Company (USA).

Results. Under our conditions total nuclear ADP-ribosylation was linear with increasing amounts of nuclei, and reached a plateau after 10 min incubation (fig. 1). Studies performed with increasing amounts of NAD showed that a plateau was reached at approximately 0.5 mM. Some variation in basal activity was observed among different experiments (e.g. control values of figs 2 and 3) which may be attributed to the fact that it is impossible to control the uniformity of strain, sex and nutrition of our dogs. The results from pulse-chase experiments indicate that this reaction is at least partially reversible, as radioactive ADP is released into the incubation medium.

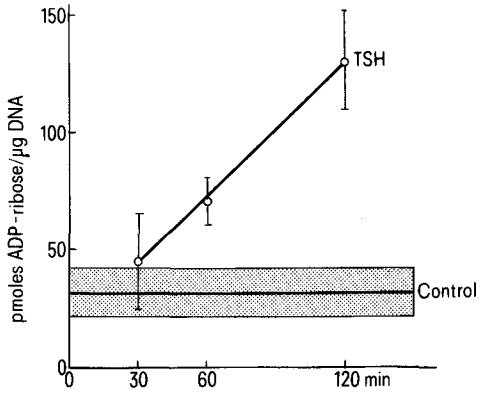

Figure 2. Time-course effect of TSH (1 mU/mL) on nuclear ADP-ribosylation. Each value is the average of triplicate determinations ± SD. Statistical analysis against the controls: 30 min TSH: NS; 60 min TSH: $p < 0.05$; 120 min TSH: $p < 0.01$.

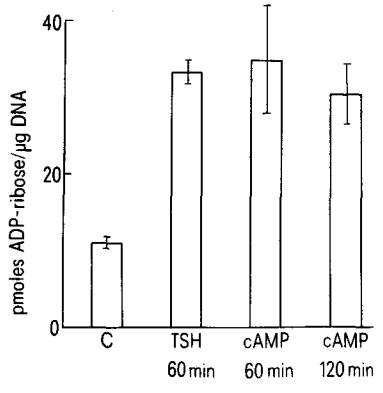

Figure 3. Comparative action of TSH (1 mU/ml) and of cyclic AMP (1 mM) on nuclear ADP-ribosylation. Each value is the average of triplicate determinations ± SD. Statistical analysis against the controls: TSH: $p < 0.001$; cAMP 60 min: $p < 0.002$; cAMP 120 min: $p < 0.005$.

The addition of TSH to the thyroid slices caused a significant stimulation of nuclear ADP-ribosylation. The table shows that 0.5 mU/ml of TSH caused a 3-fold increase of ADP-ribosylation in nuclei subsequently isolated, whilst no response was observed when slices were incubated with 0.1 mU/ml TSH. In another series of experiments, as little as 0.3 mU/ml TSH significantly increased this parameter (not shown). When the time-course of the action of TSH was examined, a slight increase in ADP-ribosylation was already evident after incubating the slices with 1 mU/ml of TSH for 30 min. Stimulation by TSH was linear up to 120 min (fig. 2). When thyroid slices were incubated with 1 mM cyclic AMP, a 3-fold increase in nuclear ADP-ribosylation was observed after 60 and 120 min (fig. 3).

Discussion. The present studies provide new evidence on the hormonal regulation of nuclear ADP-ribosylation in an eukaryotic tissue. Previous work by Filetti and Rapoport[4] has shown that TSH, at 100 mU/ml, causes an increase in total cell ADP-ribosylation. However, their experimental design involved permeabilization of the cells by hypotonic shock as NAD does not readily enter the cell. We have utilized a rather different approach: incubation of the thyroid slices under different experimental conditions, and measurement of ADP-ribosylation in nuclei isolated afterwards, thus avoiding the technical artifacts that may eventually arise from hypotonic shock. Moreover, since ADP-ribosyl-transferase activities are found in several subcellular fractions, our approach enables the localization of ADP-ribosylation to a particular fraction. In this regard it should be mentioned that Vitti et al.[14] have recently reported that TSH can stimulate ADP-ribosylation in bovine thyroid membranes. Our results demonstrate the action of TSH on nuclear ADP-ribosylation. This effect is time dependent and attained with low doses of TSH (0.3–0.5 mU/ml), which is the range of hormone concentration that elicits physiological responses, such as activation of secretion and iodination in this system[3]. As the major effect of TSH on the thyroid is to increase cellular cyclic AMP levels, we have investigated the action of this cyclic nucleotide in our system. The results herein presented show that cyclic AMP mimics the action of TSH, thus suggesting that nuclear ADP-ribosylation may follow the same pattern of regulation as other thyroid parameters[3]. Filetti and Rapoport[4] have reported an inhibitory effect of dibutyryl cyclic AMP on total cell ADP-ribosylation, which is in contrast with the stimulation that we have found at the nuclear level. This discrepancy may be due to differences in the experimental conditions, or to an action on ADP-ribosylation at another subcellular site, which may heavily contribute to the total activity measured by these authors.

Thyroid nuclear ADP-ribosylation, and its hormonal regulation, may be related to other biochemical events, such as RNA transcription[7], but this possibility remains purely speculative until further details of this reaction, and of the acceptor proteins involved, are obtained.

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Antagonistic effect of progesterone towards estradiol dipropionate-induced changes in glycogen content in uterus and vagina of P-mice

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Summary. Estradiol dipropionate induces an increase (3-fold) in the uterine glycogen content and a decrease (4-fold) in the vaginal glycogen content of Parkes (P) mice. Progesterone antagonizes this estradiol dipropionate-induced response in both the uterine and vaginal tissue. The degree of this antagonism is more pronounced in the uterus than in the vagina.
Key words. Mice; uterus; vagina; sex steroids; glycogen.

The effect of estrogen on uterine and vaginal glycogen content is well studied in a number of mammalian species. Further attempts were made to find out the effect of progesterone on estrogen-mediated response of uterine and vaginal glycogen levels in the rat and as a result contradictory findings have been reported. For instance, the combined administration of estrogen and progesterone did not produce any change in the uterine glycogen content as compared to the estrogen-treated animals[2]. In contrast, the conjoint administration of estrogen and progesterone caused a decrease in the uterine glycogen content in comparison to the condition when estrogen was given alone[3,4]. Moreover, the conjoint treatment with estrogen and progesterone produced a decrease in uterine glycogen content and an increase in vaginal glycogen content as compared to the rats which received estrogen alone[5]. This indicated the organ-specific differences in the glycogen content of uterus and vagina in response to a combined treatment with estrogen and progesterone. Therefore, the problem of estrogen-progesterone interaction seemed to be of remarkable importance. Similarly, many other investigators have also examined the glycogen level as a biochemical correlate of the process of vaginal keratinization[6,7]. These observations led us to investigate the effect of progesterone on the estrogen-induced response of uterine and vaginal glycogen contents of albino mice (P-strain).

Material and methods. There are several inbred albino strains of the common house mouse, *Mus musculus* Linn. Various strains differ in the duration and phases of the estrous cycle and in the response to exogenous female sex hormones[8]. Adult virgin female mice of the Parkes (P) strain (60–100 days old) were used in the present investigation. They are polyestrous rodents having an average 4–5 days of estrous cycle. The parental stock of P-mice was obtained from the Central Drug Research Institute (CDRI), Lucknow (India); and they were bred in the animal house of the Department of Zoology, Banaras Hindu University. All female mice were maintained with food and water ad libitum under laboratory conditions at 25 ± 3 °C and natural photoperiod (13L:11D) for 14 days prior to experimentation. They were bilaterally ovariectomized and 7 days after ovariectomy were divided into the following four groups for the treatment:

Group I (control): 0.05 ml olive oil.
Group II: 20 µg estradiol dipropionate (E-DIP).
Group III: 2 mg progesterone.
Group IV: 20 µg E-DIP+2 mg P (1:100).

Each individual received daily the above mentioned physiological doses by s.c. injection continuously for 7 days. Ampoules of hormonal injection (estradiol dipropionate and progesterone) were purchased from Ciba-Geigy Ltd., Bombay, and olive oil was used as a carrier vehicle. Animals were killed by cervical dislocation 24 h after the last injection. Uteri and vaginae were collected immediately after sacrifice from experimental and control groups for biochemical estimation. Glycogen was determined by Montgomery's[9] method, and the content determined (µg/mg wet wt tissue) using a linear standard curve for glycogen. Student's t-test was employed for statistical analysis.

Results. It is evident from the table that the administration of estradiol dipropionate to ovariectomized mice produced a 3-fold increase in uterine glycogen content while progesterone reduced the glycogen content in comparison to the control. The combined treatment with estradiol dipropionate and progesterone at a dose ratio of 1:100 resulted in a decrease in uterine glycogen content as compared to the control value. In the vagina, estradiol dipropionate caused a 4-fold decrease in the glycogen content while progesterone showed an insignificant decrease. However, the conjoint treatment with estradiol dipropionate and progesterone at 1:100 dose ratio revealed a statistically insignificant difference in the vaginal glycogen content as compared to the control value.

Moreover, the significant differences were noticed in uterine as well as vaginal glycogen content when the values of group IV

Effects of exogenous female sex steroids on the glycogen contents of uterus and vagina of P-mice

Group	Treatment	Uterine glycogen (µg/mg wet wt)	Vaginal glycogen (µg/mg wet wt)
I	Control	0.808 ± 0.056	2.213 ± 0.141
II	Estradiol dipropionate (E-DIP)	2.469 ± 0.033 ($p < 0.001$)	0.565 ± 0.008 ($p < 0.001$)
III	Progesterone (P)	0.677 ± 0.053 ($p < 0.05$)	2.060 ± 0.091 ($p > 0.10$)
IV	*E-DIP + P (1:100)	0.168 ± 0.040 ($p < 0.001$)	2.214 ± 0.050 ($p > 0.20$)

The results are expressed as mean ± SEM of 3–4 individual determinations. P values are given in parenthesis in comparison to their control values. *$p < 0.001$ vs group II.