Effect of taurine on mitogen response of human lymphocytes[1]

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Summary. Taurine selectively inhibits the phytohemagglutinin-stimulated incorporation of ^{3}H-thymidine by human cultured lymphocytes (50% inhibition by 12.5 mM taurine). Decreasing effects of taurine on Na-K ATPase activity or on calcium accumulation by lymphocytes might be responsible for its action on cell proliferation.

Taurine concentrations in some immature tissues appear to be higher than in adult tissues. In fetal brain and liver, very large concentrations of taurine are found, with 4-5-fold differences between the fetal and the mature organ[2-5]. Taurine amounts in human blood are also greater in the newborn than in the blood of children and adults[6]. The significance of these high amounts of taurine in the developing tissues is unknown; even the role of the amino acid in adult tissues remains to be elucidated. Taurine has been related to the mechanisms which regulate ionic gradients in various animal tissues. The amino acid seems to counteract ionic imbalances in tissues and body fluids[7,8], and has been reported to modify K^+ and Ca^{2+} transport [7,9-11]. Accumulating evidence has emphasized the importance of these ions in the regulation of cellular division[12,13]; compounds like ouabain which modify ionic transport were shown to be important inhibitors of the transformation of human lymphocytes[14]. The described effect of taurine on ionic fluxes, as well as some similarities between ouabain and taurine actions, prompted us to examine the effect of taurine in the transformation induced by phytohemagglutinin (PHA) in lymphocytes from cultured human whole blood, in order to assess its possible involvement in the mechanisms responsible for the control of cell proliferation.

Material and methods. Lymphocytes from human whole blood were cultured following the procedure of Arakaki and Sparkes[15]. Human heparinized venous blood (0.3 ml) was placed in 0.8 ml of the culture medium TC 199 (Difco Laboratories, Detroit, Mich.) containing 0.2 ml of heat inactivated fetal calf serum (Gibco Laboratories, Grand Island, New York). 2 mM glutamine, penicillin 100 units and streptomycin 100 µg. The cultures were maintained at 37 °C for 72 h. Phytohemagglutinin 'M' (Difco) was used at a concentration of 0.4 mg/ml. Taurine (Sigma Chemical Co. St. Louis, Mo.) at the concentrations indicated for each experiment, was added at different times during the incubation period. Other amino acids such as glycine, valine and glutamic acid, were tested for comparison under the same experimental conditions. Uptake of ^{3}H-thymidine was used as an indicator of lymphocyte transformation. 15 h before the end of the incubation 1 µCi of ^{3}H-thymidine (sp. act. 2 Ci/mmole; New England Nuclear Co. Boston, Ma.) was added to each tube and incubation continued until the 72-h period was completed. At the end of the incubation, cells were sedimented by centrifugation, fixed in acetic acid-alcohol mixture (3:1 v/v) and precipitated by cold trichloroacetic acid (TCA). The TCA-insoluble fractions were collected in glass fibre filters (Millipore, Co. Bedford, Mass.) and radioactivity was measured by scintil-

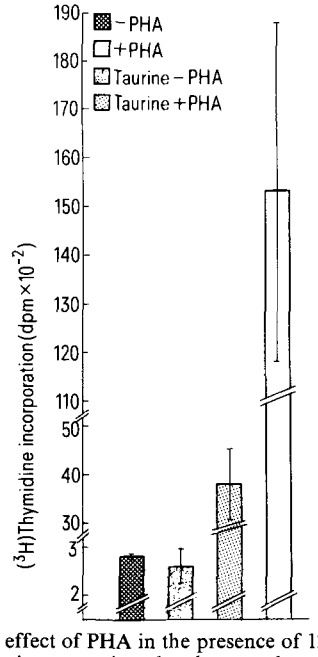

Figure 1. The effect of PHA in the presence of 12.5 mM taurine on ^{3}H-thymidine incorporation by human lymphocytes in vitro. Human blood was incubated in culture medium, at 37 °C in the presence or absence of PHA (0.4 mg/ml), during 72 h, as described in Material and methods. Taurine, when present, was added from the beginning of the incubation. ^{3}H-thymidine was added 15 h before the end of the incubation. The results are the mean ± SEM obtained in triplicate from 3 separate experiments.

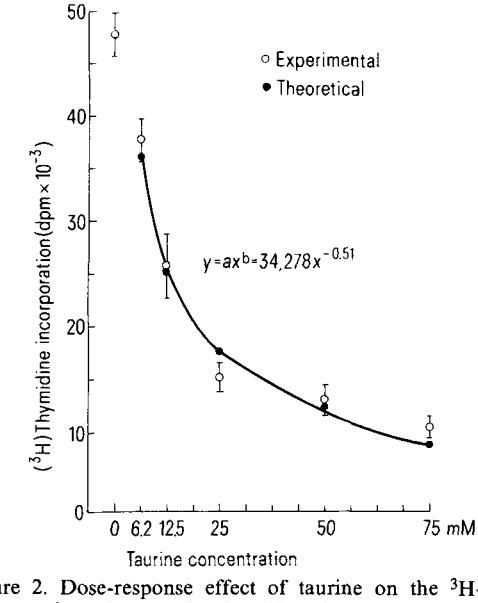

Figure 2. Dose-response effect of taurine on the ^{3}H-thymidine incorporation of PHA stimulated lymphocytes. Experimental conditions were as described in figure 1. Taurine, at the different doses used, was added from the beginning of the culture incubation (72 h). The results are the means ± SEM of 3 separate experiments. Theoretical curve was calculated by the least squares method, difference between theoretical and experimental points was proved non-significant by the χ^2 test ($p < 0.005$).

lation spectrometry. Taurine effects on Na-K ATPase and on ^{45}Ca uptake were studied in lymphocytes purified from heparinized blood by Ficoll-Hypaque (Pharmacia) density gradient centrifugation. Na-K ATPase activity was determined by measuring Pi released in presence of an ATP regenerating system[16] in an incubation mixture containing 6 mM sodium ATP, 3 mM $MgCl_2$, 120 mM NaCl, 20 mM KCl and 50 mM TRIS-acetate, pH 7.4. Calcium uptake was determined by measuring ^{45}Ca accumulation by isolated lymphocytes as previously described[11].

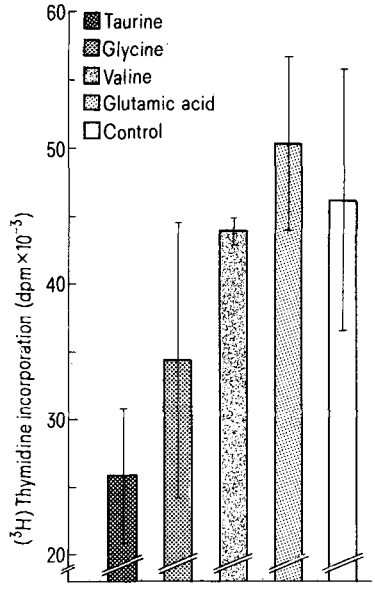

Figure 3. ^3H-thymidine incorporation in the presence of glycine, valine and glutamic acid (12.5 mM). Amino acids were added from the beginning of incubation. The results are the means ± SEM of 3 separate experiments.

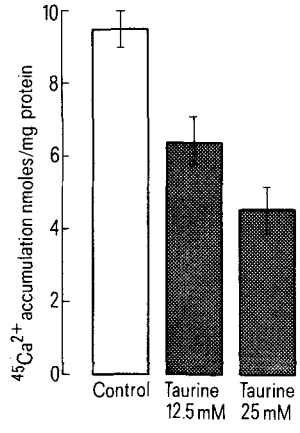

Figure 4. The effect of taurine on ^{45}Ca accumulation by lymphocytes purified by Ficoll density gradient centrifugation incubated in Krebs-bicarbonate medium, pH 7.4, at 37 °C during 5 min, in the presence of $^{45}CaCl_2$. Taurine at the different concentrations tested, was present in the medium throughout all the incubation period. The radioactivity accumulated by the cells was measured in a solubilized pellet obtained by centrifugation of aliquots of the incubation medium in a Beckman microfuge. The points are means ± SEM of 4–8 separate experiments.

Results and discussion. Human lymphocytes in vivo are cells in a quiescent, nondividing state. Mitogens like PHA are able to promote a proliferating response of the cells cultured in vitro. Figure 1 shows that PHA present in the incubation medium for 72 h stimulated 40–70 times the incorporation of ^3H-thymidine into lymphocytes. The presence of taurine at concentrations varying from 6.2 to 75 mM produced a significant decrease of the ^3H-thymidine incorporation induced by PHA. The inhibition observed corresponded to an exponential decay curve with increasing taurine doses (fig. 2). Taurine concentration required for a 50% reduction of the stimulatory effect of PHA was 12.5 mM. The effect of taurine increased as a function of the time of exposure of the cultured cells. At 12.5 mM concentration it was less than 10% after 24 h of exposure and increased to 40% after 72 h of exposure as compared with unexposed controls. The effect of taurine seems to be specific. Other amino acids tested for comparison, glycine, glutamic acid and valine, did not significantly affect ^3H-thymidine incorporation. Used at a concentration of 12.5 mM, glycine reduced this incorporation by 25% and valine by 5%; glutamic acid even enhanced it, by approximately 10%, as compared with controls (fig. 3).

The mitogenic effect of PHA has been related to an alteration of potassium fluxes probably through a modification of the Na-K activity[14,16]. In order to get some insight into the mechanism of taurine action, we have examined its effects on the ATPase activity stimulated by PHA. We observed that the mitogen produced a substantial increase in the Na-K ATPase of isolated lymphocytes (328%) and that the addition of taurine (12.5 mM) produced a decrease of about 25% on the PHA stimulated ATPase activity. (Control: 0.127 ± 0.016; PHA: 0.544 ± 0.053; PHA + taurine: 0.406 ± 0.044; $n = 4$.)

An alternative mechanism for taurine action might be related to an effect of the amino acid on calcium transport by the cells. Multiple experimental evidence supports a critical role for calcium in the initiation of the intracellular events which mediate cell activation and proliferation[12,13]. In human lymphocytes, calcium also appears to be essential for the cell transformation induced by PHA. The stimulatory effect of the mitogen is not observed in the absence of external calcium or in the presence of calcium chelators[17,18]. An inhibitory effect of taurine on calcium accumulation by brain subcellular fractions[11,19] and by sarcolemma[20] has previously been described. In the present study we observed a similar action of taurine, decreasing ^{45}Ca accumulation by isolated lymphocytes (fig. 4). These results suggest that the arresting effect of taurine could be mediated through an action on calcium availability for cell division.

Since taurine concentration in plasma and in the extracellular fluid is low, the effects of external taurine observed in the present study might reflect a pharmacological effect rather than a physiological action. However, we have observed that taurine is actively taken up by lymphocytes and the effects reported in the present study could be subsequent to its accumulation inside the cells. This possibility might involve taurine in the mechanisms regulating cell division.

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Antifertility effects of clonidine in laying hens

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Summary. Clonidine was anovulatory and markedly antigonadal in laying hens when infused for 1 week from minipump implants at daily rates of 1.08 mg per hen or greater. The ovaries of hens treated with clonidine responded to FSH injections which suggests that the antigonadal effect of clonidine resulted from a reduction in the output of gonadotropin by the pituitary. These data suggest that α_2 receptors may be important in regulating avian fertility.

There are several indications that adrenergic systems play an important role in regulating avian fertility. For example, the α-adrenergic blocking agents dibenamine[1] and phenoxybenzamine[2,3] were anovulatory in laying hens, and the catecholamine depleting agent reserpine reduced luteinizing-hormone concentrations in the plasma of cockerels as well as laying hens[4]. For *Coturnix* quail, the testicular development induced by lengthening daylight was blocked by inhibiting either tyrosine hydroxylase, the branch enzyme of catecholamine synthesis, or dopamine β-hydroxylase, whose product is norepinephrine[5]. A recent publication from this laboratory demonstrated that several inhibitors of dopamine β-hydroxylase had potent antigonadal and anovulatory activity in laying hens[6]. Since the anovulatory effect could be overcome by a single injection of luteinizing hormone releasing hormone, the hydroxylase inhibitors appeared to block fertility within the hypothalamus or elsewhere within the central nervous system. Taken together, these publications suggest that central α-adrenergic activity is required for avian fertility.

The introduction of highly specific α-adrenergic agonists and antagonists has resulted in the recent division of α-adrenergic receptors into 2 subclasses - α_1 and α_2[7]. α_1-Receptors, which always appear to be located postjunctionally, have relatively high affinity for the agonist methoxamine, while α_2-receptors, which can be located either prejunctionally or postjunctionally, have a relatively high affinity for the agonist clonidine. The major role of α_2-receptors appears to be the reduction of sympathetic outflow. Since phenoxybenzamine is a relatively specific antagonist for α_1-receptors, the work quoted above suggests that α_1-receptors play a role in avian reproduction. It was of

Table 1. Egg production and reproductive systems of hens treated with clonidine for 1 week

	Body weight change (percent of initial weight ± SD)	Ovarian wt ± SD (g)	Oviduct wt ± SD (g)	Percent of hens laying eggs on day 1	2	3	4	5	6	7
A. Oral treatment (fed)[a]										
Dose (ppm)										
0	+0±2	51±3	56±5	83	67	83	100	100	100	50
100	+5±3*	44±23	50±19	50	100	100	0	75	50	50
200	+6±4*	26±23*	37±17*	100	50	75	0	100	50	0
400	+5±4*	16±4*	22±3*	75	50	50	25	25	25	0
600	+2±2	11±5*	19±6*	50	75	75	25	25	0	0
B. Parenteral treatment (implant)[b]										
Daily dose (mg/hen)										
0	−1±2	58±10	61±9	100	100	80	60	60	100	80
0.48	+4±1*	51±9	67±10	100	100	67	100	67	100	67
1.08	+1±8	13±6*	23±5*	67	33	33	0	0	0	0
2.16	+5±5*	9±3*	23±5*	100	100	0	0	0	0	0

*Significantly different from untreated (t-test: $p < 0.05$). [a]Each treated group was of 4 hens and the untreated group was of 6 hens. [b]Each treated group was of 3 hens and the untreated group was of 5 hens.