Triiodothyronine, diluted according homeopathic techniques, modifies the programmed cell death of tadpole tail's explants

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ABSTRACT

High Dilution is a solution beyond the Avogadro limits that, in the dependence of the applied succussion elicits a suppressive or a stimulant effect on a living cell, with a consequent generation of an oscillatory dose-effect curve. According to Bonamin *et al.* [1], "Perhaps, the most enigmatic feature regarding the properties of high dilutions is the non-linearity of their effects. In several studies employing *in vivo* and *ex vivo* models, especially involving iso-endopathy, an oscillatory potency-effect curve has appeared. The first observations were initially considered as artifacts, but the repetition of this pattern in different studies involving completely different experimental models, in times and places equally different, points out to the existence of a property intrinsic to dynamized systems."

The entire process of anuran amphibian metamorphosis is under thyroid hormones control, included the complete resorption of the tadpole tail. In the present study, we had successfully established a protocol model to culture *Rana catesbeiana* tadpoles' tail tips *in vitro*. A random and blind study was performed, with the intent to prove that T3 5.10^{-24} M (10 cH) modifies the apoptosis induction of T3 100 nM in explants of *Rana catesbeiana* tadpoles' tail. 60 explants were distributed in three ways: Group A: without T3 action, at pharmacological and HD dose; Group B (test): under the action of T3 100 nM and treated with T3 10 cH (HD); Group C (control): under the action of T3 100 nM and treated with ethanol 70% unsuccussed.

After 96 hours of tissue culture, the mean of initial and final area (1.05 *vs.* 0.98 cm²) and apoptotic index of the explants from Group A were with minimal difference range and for this reason it wasn't included in the statistical study. In order to identify significant differences in the area and in the apoptotic index of the remainder explants of the 2 groups, B (test) and C (control), we used a student t-test. However, the mean initial and final explants' area from test and control groups were respectively 1.09 *vs.* 0.22 cm and 1.00 cm *vs.* 0.24 cm, with a mean reduction of 0.87 cm² and 0.76 cm², but this difference didn't achieve statistical significance ($p>0.05$). In contrast, apoptosis index was significantly higher in test than in control group 11.7 *vs.* 7.9 ($p<0.05$), with is confirmed at the table 1.

Table 1 The area (cm²) of *Rana catesbeiana* tadpoles' tail tips under the stimuli of T3 100nM, exposed to two different treatments: T3 10^a cH (test) and control solution.

Initial area (cm ²)		Final Length (cm ²)		Reduction (cm ²)		Apoptosis Index N° X / 1000 points	
test	control	test	control	test	control	test	control
0,93	0,74	0,08	0,08	0,85	0,66	15	10
1,07	0,69	0,23	0,07	0,84	0,62	18	0
0,64	0,73	0,12	0,13	0,52	0,60	20	8
0,94	0,82	0,22	0,08	0,72	0,74	25	0
1,57	1,03	0,20	0,17	1,37	0,86	19	12
0,98	0,86	0,22	0,12	0,76	0,74	0	13
1,00	0,89	0,30	0,36	0,70	0,53	5	15
1,36	0,91	0,22	0,16	1,14	0,75	12	18
-	0,96	-	0,12	-	0,84	-	7
1,08	0,71	0,18	0,11	0,90	0,60	11	0
0,96	1,09	0,11	0,23	0,85	0,86	10	20
1,27	1,27	0,25	0,19	1,02	1,08	0	10
1,16	1,32	0,35	0,78	0,81	0,54	13	2
1,08	1,21	0,14	0,40	0,94	0,81	0	10
1,04	1,03	0,22	0,28	0,82	0,75	19	5
1,09	1,26	0,17	0,18	0,92	1,08	14	5
1,08	0,90	0,19	0,14	0,89	0,76	29	3
1,11	1,41	0,23	0,58	0,88	0,83	5	5
1,22	0,95	0,17	0,17	1,05	0,78	5	8
1,12	1,25	0,50	0,39	0,62	0,86	3	6
χ 1.09	χ 1.00	χ 0.22	χ 0.24	χ 0.87	χ 0.76	χ 11.7	χ 7.9

In the 1990s, Endler *et al.* [2,3] adapted an amphibian model to study the sensitivity of *Rana temporaria* to High Dilution (HD) of thyroxin (T4) during their metamorphosis. Substances highly diluted so that no original molecule is present still have biological effects thought to be related through molecular bio-information transduced via water [2,3]. In 2003, based on this works, we published the results of the influence of an agitated highly dilution of thyroid glands decreasing the rate of *Rana catesbeiana*'s metamorphosis [4], which study reproduced Ender's experimental results, since the reproductively is one of the most serious troubles of HD research.

In the present study we detected a higher index of apoptosis in the explants with the action of T3 10 cH (HD) than the ones without this action, hence it follows that the homeopathic medicine enhance the action of T3 at pharmacological dose. It contrasts with the data obtained in former works, in with the strongly diluted substances have an opposite effect to that of mother solution. However, like mentioned previously, non-linearity is a common feature of high dilutions. Also, the previous condition of the living system seems to interfere in the outcome [1]. It is important to note that we worked with explants and not with the whole animal.

High Dilutions is not easy to study, as it requires the use of more doses (especially in the low-dose zone), often including a temporal component (measurement at various times within an experiment) and using more

subjects to enhance statistical power, and needs replication. These extra features often steer researchers to less resource-intensive and more readily definable phenomena [5]. With this intent we will repeat the experiment with a more physiological concentration of T3, something about 5 nM and with others dynamizations of T3 in HD.

In summary, an High Dilution model was developed to study the influence of T3 in a HD modifying the apoptosis of *Rana catesbeiana* tadpole tail, *in vitro*. In this model, T3 5.10^{-24} M (10 cH), added to the medium with the explants at the action of T3 100 nM, induced a high and significant ($p < 0.05$) index of apoptosis. Therefore we can reaffirm the conclusions of a great number of researches working with High Dilutions about its action. Experiments using more doses, especially in the low-dose zone, and the immunohistochemical analysis, of the expression of the Apoptosis' activators in the remaining explants will be necessary.

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Conflict of interest: authors declare there is no conflict of interest

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