

The Effect of Highly Diluted Agitated Thyroxine on the Climbing Activity of Frogs

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ABSTRACT. We studied the influence of specially prepared highly diluted thyroxine on the spontaneous tendency of juvenile frogs, which were at the end of thyroxine-controlled metamorphosis, to leave the water and climb onto land. The test dilution with a thyroxine concentration beyond Avogadro's value (dilution thyroxine D30) and the reference (dilution water D30) were prepared according to directions from the literature on homeopathy. A few drops of these solutions were added to tap water of basins containing the frogs. The frogs' climbing activities were monitored immediately after adding the solutions. The hypothesis derived from a preliminary study was that there is less climbing activity in frogs treated with dilution thyroxine D30 than in a reference group. This hypothesis was proven. Climbing activity diminished under the influence of dilution thyroxine D30, with statistical significance both in comparison to the effect of the analogously prepared solvent (dilution water D30) as well as in comparison to control observations before the start of treatment. When in a later step of observation the dilution water D30-control group was treated with dilution thyroxine D30, the diminishing effect on activity also occurred.

In various detoxification experiments with specially homeopathically (1) prepared high dilutions, organisms are first intoxicated with a high dose of a toxin (eg arsenite) followed by the addition of a highly diluted, agitated solution of the same toxin. Using this protocol, an enhanced detoxification has been observed in comparison with control on different organisms (2-4). In some respect, our model is analogous to these detoxification studies.

Further, our model depends on the following facts: Thyroxine, which is generally known for its important steering role in the metamorphosis of amphibia (5-7), applied in classical pharmacological concentrations, may provoke hyperactivity in vertebrates (8), and physiological lack of thyroxine, eg due to thyroidectomy, causes loss of activity (8). This led to the speculation that amphibia just at the end of thyroxine-controlled metamorphosis might be sensitive to a homeopathically prepared high dilution of that hormone. In 1991, we published preliminary results on the influence of an agitated high dilution of thyroxine on the climbing activity of highland frogs (9). In these blind experiments that were controlled by the Zoological Institute of the University of Graz (Prof Dr G Fachbach), an immediate significant reduction of the activity was observed under the influence of the test

dilution. For the paper presented here, this study was independently and blindly repeated in another laboratory by an independent researcher. In addition to the protocol of 1991, we have introduced a cross-over control.

In the present article, the hypothesis was studied that the test dilution had an inhibiting effect on the thyroxine-influenced climbing activity of highland frogs.

MATERIALS AND METHODS

The experiments were performed indoors in the Koralpe region of Austria by Dr W Pongratz.

Animals

A total of 130 *Rana temporaria* juveniles from a highland pool 1600 m above sea level (Koralpe, Austria) were taken at the 4-legged, tailless stage of the juvenile frog, about stage 46 according to Gosner's staging table (10). In that stage, the frogs were highly actively aiming to leave the water and go at land.

Preparation of Test Solutions

The frogs were observed under the influence of tetra-iodo-thyronine sodium pentahydrate (Sigma) specially prepared in an aqueous

solution 1:10³⁰ part of weight. The stock solution had a concentration of 1:10⁴ part of the original substance of weight; it was diluted in steps of 1:10. The diluted solution was agitated according to standardized instructions (11); at every step a sterile bottle was partly filled with the dilution with the help of a disposable pipette and was pushed down at short regular intervals (eg against a rubber impediment) to create mechanical shock. A dilution of 1:10³⁰ was chosen according to the literature on experiments with highly diluted thyroid substance (12). The test solution prepared in this way was called dilution thyroxine D30. As a reference solution the solvent (pure distilled water) was prepared in an analogous way (dilution water D30). The solution set for the experiments was coded at the Boltzmann Institute fur Homöopathie in Graz. The set was applied blindly.

Treatment

Dilution thyroxine D30, as well as the control dilution water D30, was always applied by putting 2 drops of the respective dilution into 0.5 L water of the basins containing the frogs, followed by gentle shaking of the basins.

Observation of Climbing Activity

White plastic basins (1 x w x h : 34 x 22 x 14.5 cm, Firma Miraplast, Austria) were each filled with 0.5 L of pure lake water. The juvenile frogs spontaneously climbing out of the water and up the walls of the basins were counted. In some cases, photos and a video were prepared as documentation. The climbing activity of juveniles in the course of 3 min was investigated by always comparing 2 groups of frogs in 2 different basins. All the frogs that climbed up the walls to a certain height were put back into the water before measurement; then both basins were shaken gently to provide the same starting conditions for the 2 groups. The number of climbers that had brought at least 4 of their 5 'extremities' (including the stump of the tail) out of the water was counted 1, 2 and 3 min after last shaking the basins. This experiment was repeated 5 times within 20 min. In the control experiment (step A), climbing activity was measured as described above without any treatment. In step B, the frogs were treated with dilution thyroxine D30 or dilution water D30. Immediately after this treatment, climbing activity was observed as above (blind experiment). In step C (cross-over treatment), the water D30 group from step B was treated with dilution thyroxine D30 and vice versa. An immediate observation was not performed. After 2 d, climbing activity was observed for 20 min as above. With regard to the fact that the 2 groups treated with dilutions thyroxine D30 and water D30, respectively, have been differentiated at the end of step B by the (blinded) researcher, the subsequent step C can only be described as an open experiment.

Further Conditions

Indirect light was used. Room temperature was kept constant. Any external shocks were avoided. Five small sub-experiments were performed. Two basins were always used for treatment with dilutions thyroxine D30 and water D30, respectively. For each sub-experiment the frogs were added to the 2 basins in the following manner before the dilutions were applied. All frogs were kept in 1 container and were submerged with water in order to prevent them from climbing. Then, with subsequent submergion, always 1 frog was taken out of the water and was put into 1 basin, then a second frog was taken out and put into the other basin, then a third frog was placed in the first basin, a fourth into the second basin and so on until each basin contained 13 frogs. It was decided at random which group of frogs would be treated with which (blinded) test solution. This procedure made for a total of 130 frogs and 650 observed cases/step, respectively. The positions of the basins were changed regularly to avoid any influence of spatial factors. If a frog had reached the edge of the basin, it was put back using a disposable wooden stick.

Evaluation of Data

The cumulative frequency of climbers (Fc) was compared to that of frogs remaining in the water. For every one of the 5 successive repetitions of the observation, the cumulative frequencies of climbing attempts in the 2 groups were evaluated as a 4-field table by a Chi-square test at the specific measuring points in time. This differentiated evaluation was made for step 1 (reference), for step 2 (treatment) and for step 3 (cross-over treatment). In order to investigate reliability, the single repetitions of the climbing observation were compared by Wilcoxon test at the measuring points in time. Furthermore, in order to get a survey over the data, the numbers of climbing frogs were added up for each step (reference, treatment and cross-over treatment) at the 3 different times (1, 2 and 3 min) after the start of the respective experiment.

RESULTS

Five experiments were performed with a total of 130 frogs. In Fig 1, the data from the respective repetitions 1-5 were pooled for each step. In Fig 1A, the 2 curves give the increase of climbing activity Fc for frogs assigned for later treatment with dilution thyroxine D30 or dilution water D30. In this step A, before treatment, the increase of Fc is practically identical for both groups at all time points. In Fig 1B, the curves give the increase in step B, when dilutions thyroxine D30 and water D30 were added and observations were repeated immediately 5 times. The Fc values for the dilution thyroxine D30 group were 5-10% below those for the group treated with dilution water D30. In Fig 1C, the 2 curves show the increase after the cross-over treatment. In the 5 repetitions of the climbing observation, the Fc values for the frogs

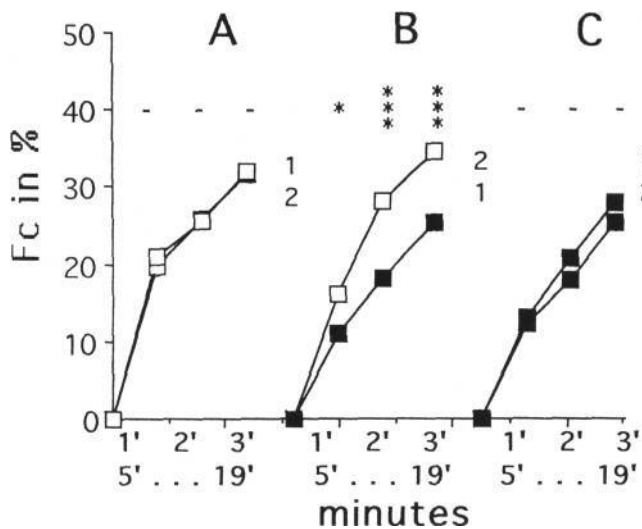


Figure 1. The influence of dilution thyroxine D30 on the climbing activity of juvenile highland frogs. Ordinate: Cumulative frequency of climbers Fc. 100% refers to the whole number of observed climbers in the 5 successive repetitions of each step (A, B, C) of the experiment (325 cases in each step). Abscissa: The measuring points in time for the 5 repetitive observations; min 1-3, 5-7, 9-11, 13-15, 17-19, pooled data. Step A = climbing activity before treatment; cumulative frequency of climbers assigned for later treatment with dilution thyroxine D30 (curve 1) and with dilution water D30 (curve 2). Step B = immediately after treatment with the dilutions; black squares, frogs treated with thyroxine D30; white squares, frogs treated with water D30. Step C = 2 days after crossover treatment; all frogs are finally treated with the dilution thyroxine D30. For further details, see text.

treated with dilution thyroxine D30 + dilution water D30 until step C remained below those of step A, whereas the Fc values for the frogs treated with dilution thyroxine D30 in step C only decreased 3-10%.

Figure 2 is a more detailed description of step 2 (survey in Fig 1B). It shows the Fc values for dilution thyroxine D30 and for dilution water D30 immediately after the first treatment during the repetitive observations 1-5. The Fc values for dilution thyroxine D30 frogs are below those for reference frogs in all 5 repetitions. This difference is statistically significant (P between <0.05 and <0.001) for most, but not all repetitions. When the 5 repetitions were compared by a Wilcoxon test, P was 0.043 at the first, 0.068 at the second, and 0.042 at the third min.

DISCUSSION

The results observed in this study refer to juvenile highland frogs at the end of metamorphosis. They show there are differences in spontaneous climbing activity between frogs treated with dilution thyroxine D30 as compared to frogs treated with dilution water D30. Dilution thyroxine D30 slowed down activity significantly. Adding

a few drops of dilution thyroxine D30 to the water in the basins induced a slowing down of the rate of spontaneous climbing activity in the amphibia. This was consistent with the study reported by an independent researcher (9) that used a total of 78 frogs. The dilutions used then were checked for contaminations by T_4 , T_3 , TSH and iodine independently and blindly at university institutes (Doz Dr G Passat, Prof Dr G Knapp). Hormone active contaminations were excluded up to the accuracy of measurement, ie the probes were free from contamination within the range of classical pharmacological doses. In the study in step A, before treatment, the increase of climbing activity Fc was practically identical for the 2 groups at all time points. In step B, dilutions thyroxine D30 and water D30, respectively, were added and the observation was repeated immediately. In these follow-up repetitions of the climbing observation, the Fc values for thyroxine D30 frogs were significantly below those for the reference group (9). Using a different experimental design, an inhibitory effect of dilution thyroxine D30 on the climbing activity of highland frogs has also been shown in a recent study performed by 3 researchers from Graz and 1 from Utrecht, Netherlands (Endler PC, Pongratz W, van Wijk R et al in Endler PC, Schulte J eds: *Ultra High Dilution, Physiology and Physics*. Kluwer Academic Publishers, Dordrecht, 1993, in press).

This tendency to reduce activity is opposite to that known for thyroxine in pharmacological doses (8), but is consistent with the results of our earlier studies on the effect of dilution thyroxine D30 on the transition from the 2-legged to the 4-legged stage in the metamorphosis of frogs (13,14). In these studies in 2 laboratories in Graz and in 1 laboratory in Utrecht, an inhibitory effect of dilution thyroxine D30 compared to dilution water D30 was shown (13,14). This inhibitory effect was also seen in recent controlled experiments using another method

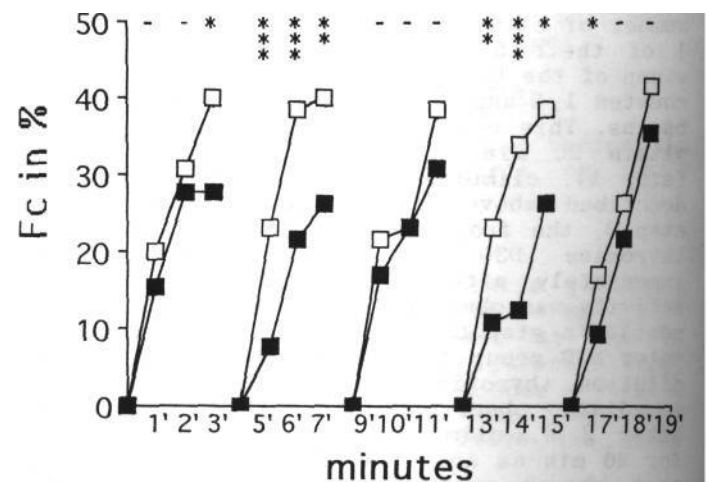


Figure 2. Detailed description of step B (pooled cumulative frequencies in Fig 1B). 1005 refers to 65 frogs. --not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001. For further details, see Fig 1 and text.

in a laboratory in Graz, in another laboratory in the Koralpe region and in 1 laboratory in Vienna (Endler PC, Pongratz W, van Wijk R et al in Endler PC, Schulte J eds: Ultra High Dilution, Physiology and Physics. Kluwer Academic Publishers, Dordrecht, 1993, in press).

There is a parallel between detoxification studies (2-4) and our experiments (13). In detoxification experiments, organisms are first intoxicated with a high dose of a toxin followed by the addition of a low dose or a highly diluted agitated solution of the same toxin. In such studies, an enhanced detoxification has been observed in comparison with controls using different organisms (2-4). In our experiments the frogs were not intoxicated with thyroxine, but the parallel to detoxification experiments exists in an enhanced susceptibility to thyroxine in the experimental animals. When thyroxine is applied in pharmacological doses, a stimulatory effect on metamorphosis (5-7) and on the activity of vertebrates (8) has been described. In contrast, when a highly diluted agitated solution of thyroxine was added, the speed of metamorphosis of tadpoles and the spontaneous climbing activity of juveniles decreased. This is in agreement with other observations described in the homeopathic literature, such as the reduction of hayfever symptoms when highly diluted agitated grass pollen was administered to patients suffering from hayfever (15). These patients were highly susceptible to grass pollen. At present, we have no insight into the physiological processes affected by homeopathically prepared dilution thyroxine D30.

The mechanism of information storage in the test substance is also unknown, but several hypotheses have been postulated. The most common states there is a coherent interaction between dipoles of the solvent water and the electromagnetic field of molecules of the diluted mother substance, including a permanent polarization of the water, which thus becomes coherent (16). The analogy to the coherence of a laser is used, but in water the coherence is in the ground state (16,17). The idea was raised that in the process of agitation of the diluted mother substance, thyroxine-, the water in the immediate environment of the biological molecule has a certain capacity as an agent of transmission. According to Benveniste (personal communication, Kiev 1992; 18-20) the radiation fields of the charged thyroxine molecule might generate a permanent polarisation of thousands of water molecules in its environment (14). During the homeopathic agitation process, this perimolecular water is then separated from the thyroxine molecule, but continues to carry its message. (For further discussion, see Endler PC, Schulte J eds: Ultra High Dilution, Physiology and Physics. Kluwer Academic Publishers, Dordrecht, 1994, in

press, and 21).

The results described in this paper show that a homeopathic dilution of thyroxine slows down a process that is enhanced by comparatively high blood and tissue level (22) of that substance. These observations may be of importance when considering the mechanism of action of high dilutions in homeopathic toxicology.

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