

Modulation of the platelet serotonin transporter by thermal balneotherapy: a study in healthy subjects

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Abstract. – Background: Although the beneficial effects of balneotherapy have been recognized since a long time, a few information is available on the biological mechanisms underlying them and the subjective feelings of increased well-being and mood.

Aim: The links between the serotonin (5-HT) system and mood prompted us to investigate the 5-HT platelet transporter (SERT), which is considered a reliable, peripheral marker of the same structure present in presynaptic neurons, in 30 healthy volunteers before (t0) and 30 minutes after (t1) thermal balneotherapy with ozonized water, as compared with a similar group who underwent a bath in non-mineral water.

Materials and Methods: The SERT was evaluated by means of the specific binding of ³H-paroxetine (³H-Par) to platelet membranes. Equilibrium-saturation binding data, the maximal binding capacity (Bmax) and the dissociation constant (Kd), were obtained by means of the Scatchard analysis.

Results: The results showed that, while Bmax values did not change in both groups, the Kd values decreased significantly at t1 only in those subjects who bathed in ozonized water.

Conclusions: The results of this study, while showing a decrease of the dissociation constant (Kd) which is the inverse of affinity constant, of ³H-Par binding to SERT in all subjects after balneotherapy and not in those bathing in normal water, suggest that SERT modifications may be related to a specific effect of ozonized water and, perhaps, also to the increased sense of well-being.

Key Words:

Serotonin, Serotonin Transporter, Platelets, Balneotherapy, Well-being, Mood.

Introduction

Balneotherapy, also known as spa bathing, spa therapy or thermal therapy, has been used since the Roman age as a non-conventional medical treatment first in Europe and Middle-East countries rich in mineral waters and then in USA. Balneotherapy involves the immersion of the subject in mineral baths or pools and shows beneficial properties in different disorders with no risk or side-effects, particularly, in pain relief and change of the intraarticular temperature in reumatologic disorders, such as fibromyalgia, reumatoid arthritis and osteoarthritis, and in patients with low back pain¹⁻³. Balneotherapy has also been employed in the treatment of psoriasis and atopic dermatitis with high rates of response⁴, and in chronic obstructive pulmonary diseases, for its vasodilating effects on vessels of bronchial mucose which can improve its trophic state and increase the number of secretory IgA and the muco-ciliary activity⁵. Interestingly, thermal treatment seems to improve heart-failure-related symptoms and quality of life in patients with mild chronic heart failure by enhancing cardiac and endothelial functions⁶; furthermore, in subjects with the so-called lifestyle-related diseases, such as hypertension, hyperlipidemia, diabetes mellitus, obesity, and smoking, where the vascular endothelial function is impaired too, thermal therapy seems to be promising⁷. However, the mechanisms by which this broad range of diseases are improved by balneotherapy have not been fully clarified, although they probably include thermal, mechanical, immunomodulatory and chemical effects. Similarly, no comprehensive explanation is given for the subjective effects of balneotherapy which include relaxation, a sense of well-being and a decrease of stress

Abbreviations

5-HT = serotonin

SERT = serotonin transporter

sensations, which have been related to changes of different hormones, cortisol or endogenous opiates⁸⁻¹⁰.

Different data show that the serotonin (5-HT) system is involved in the regulation of mood, affect, anxiety and impulsivity¹¹. In the past few decades, much attention has been focused on the specific protein which promotes the 5-HT reuptake into the pre-synaptic terminals, once released in the synaptic cleft, the so-called 5-HT transporter (SERT) which is the main target of antidepressants, drugs used in a different neuropsychiatric disorders and even in pain syndromes^{12,13}.

Since blood platelets and neurons share a similar reuptake system of 5-HT, platelet SERT has become an useful peripheral model of presynaptic serotonergic activity¹⁴, particularly after the demonstration of its identity with the same structure expressed in the central nervous system¹⁵. Several reports have demonstrated alterations of the SERT in neuropsychiatric disorders and conditions, irrespective of the diagnoses^{16,17}, which have been linked to dimensions or psychic states reflecting modifications of the serotonergic activity which are not always dysfunctional and might even have a strong adaptive values, when occurring in specific moments of the life^{18,19}.

Given the possible relationships between the 5-HT system, mood changes and the easiness to obtain the platelet SERT, the present study aimed at evaluating this structure in healthy subjects before and after balneotherapy treatment in Na⁺ Cl⁻ ozonized water of Montecatini Terme (Pistoia) (water "Rinfresco", R.f. at 180°C 4.0 g/l), as compared with a similar group of subjects after bathing in non-mineral water. The SERT was evaluated through the changes of binding parameters of ³H-paroxetine (³H-Par) which is considered the ligand of choice for labelling it²⁰⁻²².

This study represents the extension of another one of our group published previously²³.

Subjects and Methods

Subjects

Thirty healthy drug-free volunteers of both genders (15 men and 15 women, between 24 and 50 years of age, mean±SD: 39.4±8), recruited amongst medical and nursing staff at the Montecatini spa (Italy), were included in the study. They had no family nor personal history of any major psychiatric disorder, as assessed by a psychiatric interview, carried out by a senior psychiatrist (DM).

All subjects were free of any physical illness, as documented by a general check-up and by the normal blood and urine tests.

All were psychotropic drug-free, none were heavy smokers and none belonged to HIV-risk groups.

All individuals were subjected to balneotherapy treatment in alcalinic salso-sulphidric ozonized water of 36°C, performed in a single small pool for 20 minutes. This session was followed by a rest period in which all were wrapped in a hot cotton cloth, in clinostatic position, for an analogous length of time in a relaxing room at a temperature of 20° C.

They were compared with a similar group of healthy volunteers (15 men and 15 women, between 24 and 54 years of age, mean±SD: 40.2±7.4) who were subjected to bathing in non-mineral water of 36° C, under the same conditions.

The subjective feeling of well-being was assessed by a questionnaire, not validated as yet, which was set up for this purpose, consisting of 8 adjectives to measure changes of mood (depressed, very sad, sad, not sad/not happy, serene, happy, very happy, euphoric). At t₀, 18 subjects of the group bathing in mineral water rated themselves as "serene", 6 "not sad/not happy", 6 "happy", 15 of the other group rated themselves as "serene", 7 "not sad/not happy" and 8 "happy".

They all gave their informed written consent to participating in the study which was approved by the Ethics Committee of Pisa University.

Platelet Separation

Venous blood (30 ml) was collected from all subjects 10 minutes before (t₀) and after (t₁) 30 minutes from the balneotherapy treatment or the bath in normal water. A subgroup of subjects (n. 4, 2 M and 2 F) of the first group were re-tested after one week (t₂).

All samples were taken between 8 and 9 a.m., during one week of March, in order to avoid the possible interference of circadian or seasonal rhythms, respectively and gently mixed with 1 ml of anticoagulant 0.15 M EDTA.

Platelet-rich plasma was obtained by low-speed centrifugation (200 x g, for 20 min, at 22° C). Platelets were counted automatically using a flux cytometer (Cell-dyn 3500 system, Abbott, Milan, Italy).

Platelets were precipitated by centrifugation at 10,000 x g for 10 min at 4°C and the ensuing pellets were then stored at -80°C until the assay, which was performed within a week.

³H-Par Binding

The ³H-Par binding was carried out according to Marazziti et al¹⁶. The incubation mixture consisted of 100 μ l of platelet membranes (50-100 μ g protein/tube), 50 μ l of ³H-Par (Perkin-Elmer Life Science, Milan, Italy: 19.1 Ci/mmol) at six concentrations ranging from 0.01 to 1 nM and 1.85 ml of assay buffer (50 mM Tris HCl, 120 mM NaCl, 5 mM KCl, pH 7.4). Specific binding was obtained as the binding remaining in the presence of 10 μ M fluoxetine as a displacer. All samples were assayed in duplicate and incubated at 22°C for 1 hour. The incubation was halted while adding 5 ml of cold assay buffer. The content of the tubes was immediately filtered under vacuum through glass fibre filters GF/C and washed 3 times with 5 ml of assay buffer. Filters were then placed in vials with 4 ml of scintillation cocktail (Ready Safe scintillation cocktail, Beckman Coulter, South Kraemer Brex, CA, USA) and radioactivity was measured by means of a beta-counter (Packard LS 1600, Packard, Petaluma, CA, USA). Proteins were measured according to the method of Peterson²⁴.

Data Analysis

Equilibrium-saturation binding data, the maximum binding capacity (Bmax, fmol/mg protein) and the dissociation constant (Kd, nM), were analysed by means of iterative curve-fitting computer programmes EBDA²⁵.

The differences between the Bmax and Kd values at the assessment times were assessed by

non-parametric Wilcoxon test, and those between the two groups were analysed by means of the Mann-Whitney test. The possible effects of age and sex on biological parameters were investigated by the analysis of covariance, while the correlations between variables were explored using Pearson's method, all with the computer software Statview 5 for Macintosh, 1992. The results are expressed as mean \pm SD. Values $p < 5$ are considered statistically significant.

Results

Age or sex did not affect Bmax and Kd values of ³H-Par binding to platelet membranes.

The Bmax values (mean \pm SD) of the subjects who bathed in mineral water were 1105 \pm 270 and 989 \pm 246 fmol/mg proteins at t0 and t1, respectively. The Kd values (mean \pm SD) were significantly higher at t0 than at t1 (0.052 \pm 0.015 and 0.032 \pm 0.008 nM, respectively (Z= -3.447 $p = 0.001$) (Figure 1).

The Bmax values (mean \pm SD) of the subjects who bathed in non-mineral water were similar to those of the first group (1295 \pm 214 and 1184 \pm 370 fmol/mg proteins at t0 and t1, respectively), while their Kd values (mean \pm SD) were similar at t0 and t1 (0.056 \pm 0.03 and 0.06 \pm 0.008 nM at t0 and t1 respectively). At t1, the Kd values of the first group were significantly lower than those of the other subjects (Z= -3.027, $p = 0.002$) (Figure 1).

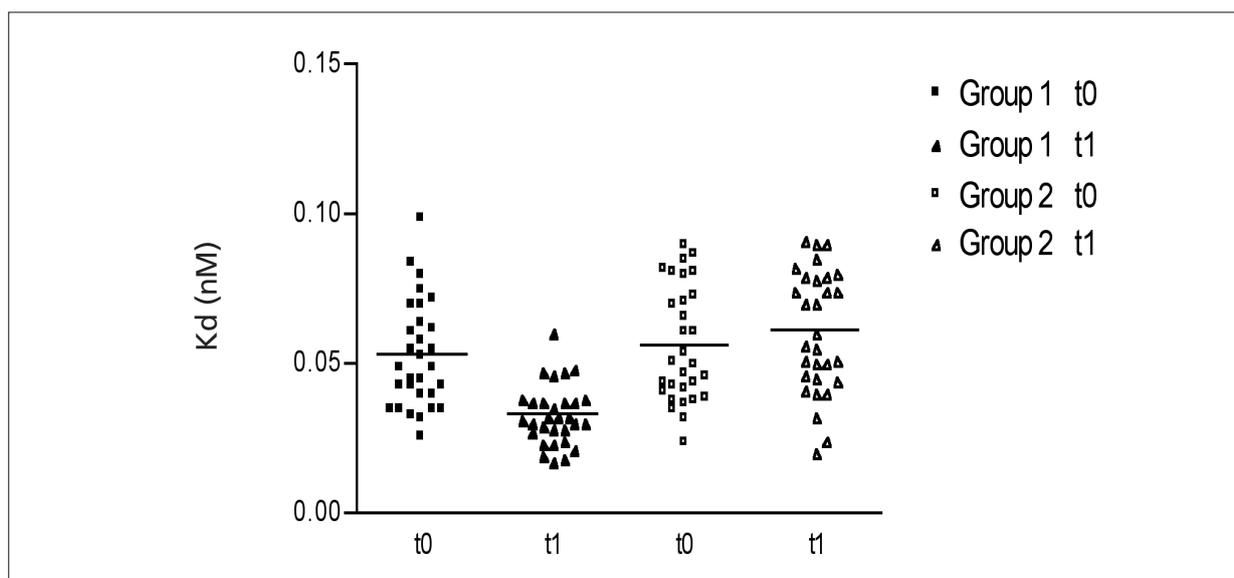


Figure 1. Scattergram of Kd values of subjects bathing in mineral water (group 1) and non-mineral water (group 2) at t0 and

All the subjects of the first group showed an increase of the mood, while rating themselves as "happy" or "very happy", while the other did not show any change.

Discussion

The results of our study revealed a significant change of the Kd, which is the inverse of the affinity constant, of the ³H-Par binding to platelet SERT following a balneotherapy treatment in alcalinic salso-sulphidric ozonized water. In fact, the Kd values of the subjects immediately after the thermal treatment (t1) were significantly lower than at baseline. These data suggest that the affinity of the SERT to its ligand increased after the thermal session which is commonly used in the treatment of peripheral vascular diseases, different dermatologic conditions, pain syndromes and a variety of medical illnesses^{3,4,26,27}. Moreover, a subjective sensation of increased mood was reported by all the subjects included in the study, although assessed by a non-specific instrument. The modification of the SERT seems to persist and to be more marked after a week from the treatment, however, the small number (n. 4) of individuals examined at that time did not permit to perform reliable statistical analyses.

The overall findings, therefore, suggest that the affinity of paroxetine for the SERT would increase following balneotherapy, that is to say, a lower amount of ligand seems to be required to obtain the protein saturation. Although it is still premature to conclude that these changes are directly related to the spa treatment, the data gathered in healthy subjects bathing in non-mineral water seems to support this notion, as they did not show any modification of the SERT parameters, or of the subjective feelings of well-being: they only reported to feel more relaxed. It, thus, seems that it is quite different to bath in mineral or in non-mineral water, at the level of both platelet SERT and individual feelings. This is in disagreement with a paper showing a similar reduction of whole blood 5-HT in subjects undergoing a meditation session or resting²⁸. The Authors concluded that the serotonergic changes observed might represent a marker of general rest and not of the relaxation following meditation. The disagreement with our results may be due to the different serotonergic parameters examined, as well as to the type of "challenge" used. How-

ever, both Solberg et al.'s and our data support the hypothesis that the serotonergic system is particularly flexible and can fastly modify its function following emotional stimuli.

The decreased Kd observed in the subjects after balneotherapy may be related to changes in the intrasynaptic concentrations of 5-HT, which might trigger or be secondary to change in other systems, such as the immune one. Interestingly, changes of inflammatory mediators following balneotherapy have been reported in patients suffering from fibromyalgia syndrome²⁹, a condition characterized also by alterations of platelet SERT³⁰. Future studies will be directed to a more exhaustive evaluation of the platelet SERT which will include the measurement of the reuptake velocity of 5-HT, in order to establish whether the SERT changes can be used as a parameter to assess the subjective changes of some psychological conditions.

Conclusions

In conclusion, the results of the present study support the notion that balneotherapy may influence the platelet SERT and improve the subjective feelings of well-being: further researches are, however, needed to establish the biochemical correlates of the empirically-recognized mood enhancing properties of spa treatments.

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