

PARTICULAR INHIBITORY ACTION OF LOW CONCENTRATIONS OF METHADONE ON PERIPHERAL T LYMPHOCYTES

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Abstract

We investigated the *in vitro* effects of methadone on the activation/proliferation capacity of mononuclear cells from healthy subjects, heroin addicts on methadone substitution therapy and rheumatoid arthritis patients, in order to reveal new aspects regarding immune – nervous systems interaction.

Lymphocyte activation/proliferation was measured as uridine uptake by the resting and polyclonally activated lymphocytes and constitutively proliferating cells with immune characteristics.

We showed that low concentrations of methadone (20ng/mL) exerted *in vitro* an immunomodulatory action on T lymphocytes from healthy subjects and from heroin addicts, whilst being immunosuppressive for T cells from rheumatoid arthritis patients.

A particular *in vitro* inhibitory action of 20ng/mL methadone on B lymphocytes was noticed only in methadone-treated addicts.

Results suggested that T lymphocytes are particularly sensitive to low concentrations of methadone and that desensitization is not traceable. Results might be further translated to inflammatory diseases aiming to control pain and inflammation simultaneously.

Rezumat

Studiul a avut ca obiectiv investigarea efectului *in vitro* al metadonei asupra capacității de activare/proliferare a celulelor mononucleare provenite de la: subiecți sănătoși, pacienți dependenți de heroină aflați în tratament de substituție cu metadonă și de la pacienți cu artrită reumatoidă. Studiul a avut drept scop elucidarea unor noi aspecte privind corelația sistem imun - sistem nervos.

Activarea/proliferarea limfocitară a fost evaluată prin metoda incorporării uridinei de către limfocite neactivate, limfocite activate policlone sau de către celule constitutiv proliferative cu caracteristici imune.

Studiul a relevat faptul că *in vitro*, concentrații mici de metadonă (20ng/mL) au o acțiune imunomodulatoare asupra limfocitelor T provenite de la subiecții sănătoși și de la dependenții de heroină, pe când asupra limfocitelor T provenite de la pacienții cu artrită reumatoidă au o acțiune imunosupresoare.

Asupra limfocitelor B, 20ng/mL metadonă au manifestat o acțiune inhibitorie *in vitro* doar în cazul dependenților aflați în tratament substitutiv cu metadonă.

Rezultatele sugerează faptul că limfocitele T sunt sensibile în special la concentrații mici de metadonă, iar desensibilizarea receptorilor nu a fost detectată. Ulterior, rezultatele pot fi aplicate în cazul afecțiunilor inflamatorii, în scopul controlării simultane a durerii și inflamației.

Keywords: methadone, T lymphocyte, lymphocyte proliferation

Introduction

Extensive crosstalk between the immune and the nervous systems takes place, especially at the level of opioids and their receptors [5]. Opiates interfere with cell-mediated immune responses by acting directly on opioid receptors μ , κ and δ , identical to those expressed by the nervous system [7]. Most of the studies regarding the immunological action of opiates were focused on heroin, but rather conflicting results were obtained, showing both decreased and increased T cell responses [2,9]. Moreover, little is known regarding the immune impact of methadone which is also a μ opioid receptor agonist. Therefore, we conducted a study on the effect exerted *in vitro* by methadone on the activation/proliferation ability of peripheral lymphocytes from heroin addicts before and during methadone substitution therapy. Cells from normal subjects and rheumatoid arthritis patients were also studied.

Materials and Methods

Chemicals. Biocol, RPMI1640 cell culture medium, fetal bovine serum, antibiotic-antimycotic solution were obtained from Promega Corporation. Methadone hydrochloride, phytohemagglutinine (PHA) and pokeweed mitogen (PWM) were purchased from Sigma-Aldrich. Tritium-labeled uridine was kindly provided by the Institute of Physics and Nuclear Engineering "Horia Hulubei", Măgurele, Romania.

Human subjects. We mainly investigated long-term heroin addicts (n=8) volunteering for substitution therapy with methadone (a medium dose of 50 mg/day), 18-35 years old, monitored at the Center for Evaluation and Treatment "St. Stelian" Bucharest, Romania. Patients were tested before methadone therapy onset and 6 months later. Age-matched normal healthy subjects (n=13) were tested for normal immune *in vitro* reactivity to methadone. A preliminary study was developed on rheumatoid arthritis (RA) patients (n=5) with active disease, on methotrexate therapy, monitored at the Research Center of Rheumatic Disease, Sf. Maria Hospital, Bucharest, Romania. The study was approved by the Ethical Committees of

the Center and Hospital and was carried out in accordance with the Declaration of Helsinki [10].

Biological samples. Mononuclear cells were isolated from peripheral blood collected on lithic heparin by centrifugation in density gradient (Biocol, Promega, $d=1.077$) [1]. Cells were counted in a Burker-Turk chamber. Cellular viability, scored by the Trypan blue exclusion test, exceeded 98%. Mononuclear cells were cultured for 72h in the presence of methadone and lectinic mitogens specific for T lymphocytes (phytohemagglutinine, PHA) or B lymphocytes (pokeweed mitogen, PWM).

The lymphoblastic cell line Jurkat was obtained from ECACC (UK) and was maintained according to the protocol provided by the manufacturer.

Uridine uptake. The proliferation/activation capacity of peripheral lymphocytes was assessed as uridine uptake *via* the alternative nucleotide biosynthesis pathway, reflecting RNA synthesis [3]. Uridine incorporation by isolated mononuclear cells, non-stimulated or *ex vivo* activated with lectin mitogens (10 μ g/mL PHA or 2.5 μ g/mL PWM), was measured by the tritium-labeled uridine incorporation test [6]. Briefly, triplicate test samples (200 μ L final volume), containing mononuclear cell suspension (0.2×10^6 cells), were incubated in absence or presence of mitogens and/or methadone, in 96 well plates, for 72h at 37 $^{\circ}$ C, in 5% CO $_2$ atmosphere. Six hours prior to harvesting, cell cultures were labeled with 1 μ Ci tritium-labeled uridine (21 Ci/mmol). Cells, harvested on glass filters, were measured for radioactivity in scintillation liquid, using a Canberra-Packard beta-counter (PerkinElmer Life and Analytical Science). Results were expressed as pulses/min (ppm).

Based on radioactivity readings, the *in vitro* effect of methadone was calculated according to the formula:

$$Effect = \frac{(sample) + methadone}{(sample) - methadone}$$

Statistical analysis. Individual data were statistically processed as mean $\pm$ standard error of the mean.

Results and Discussion

Using mononuclear cells isolated from normal healthy subjects ($n=13$) we investigated the effect exerted *in vitro* by methadone (2-20000ng/mL) on uridine uptake by lymphocytes, in order to assess *in vitro* immunologically active concentrations of the drug. Results (Figure 1) showed that 200ng/mL methadone, which is a concentration equivalent to that found in plasma of patients on methadone substitution therapy [4], had

no statistically significant effect on uridine uptake by the resting and proliferating cells.

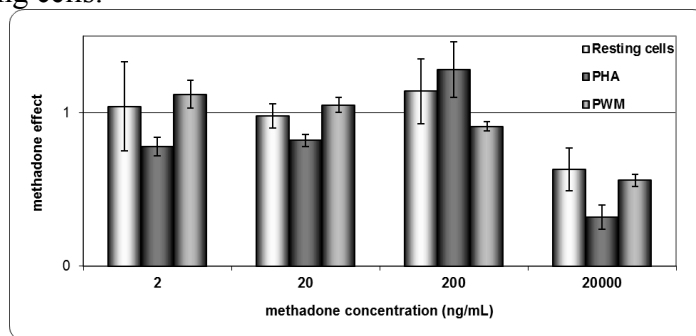


Figure 1

The *in vitro* effect of methadone on tritium-labeled uridine uptake by the resting, PHA- or PWM-activated lymphocytes from normal healthy subjects (n=13).

These *in vitro* results were sustained by our *in vivo* findings that T and B lymphocytes from patients on methadone substitution therapy for 6 months showed *ex vivo* similar ability to incorporate uridine as lymphocytes from normal healthy subjects (Figure 2).

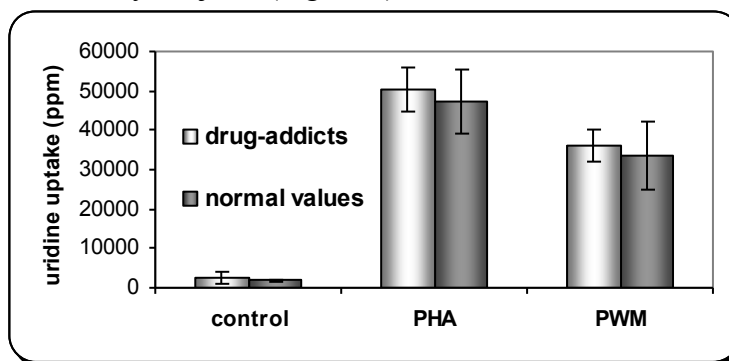


Figure 2

Ex vivo uridine uptake by lymphocytes from drug addicts after 6 month substitution therapy with methadone (n=8) vs. normal subjects. Cells were experimentally activated with PHA or PWM *in vitro*.

Lower concentrations of methadone (2 and 20ng/mL) proved to inhibit preferentially uridine uptake by PHA-activated normal T lymphocytes, but not by the resting cells or PWM-activated B lymphocytes (Figure 1). As such, 20ng/mL methadone exerted *in vitro* an immunomodulatory action on T lymphocytes, whilst B lymphocytes were rather insensitive to methadone *in vitro*. Very high concentrations of methadone (20μg/mL) drastically decreased *in vitro* uridine uptake (p<0.05)

regardless of the cellular activation status (Figure 1), thus indicating immunosuppressive effects confined to proliferating T lymphocytes.

We further investigated whether these effects were correlated with cellular proliferation. Therefore, we investigated the effects exerted *in vitro* by methadone on constitutively proliferating lymphoblastic cell line Jurkat presenting T lymphocyte characteristics (Figure 3). We found that indeed a dose of 20ng/mL methadone had the ability to inhibit uridine uptake by actively proliferating T lymphocytes.

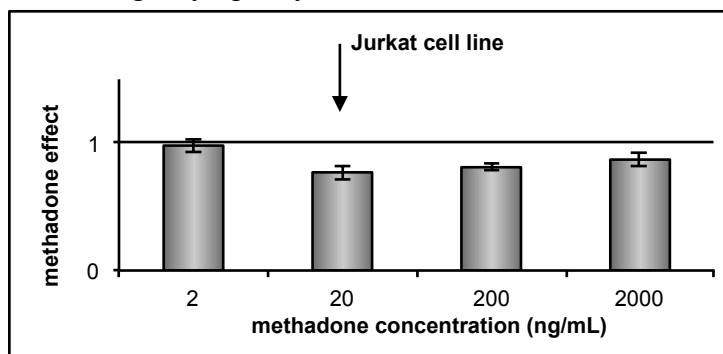


Figure 3

The *in vitro* effect of methadone on tritium-labeled uridine uptake by Jurkat cells.

Based on our results on normal healthy subjects, we further investigated the *in vitro* effect of methadone at immunologically active concentrations (20ng/mL and 20μg/mL) on lymphocytes from heroin addicts, before (Figure 4) and 6 months after the onset of methadone substitution therapy (Figure 5).

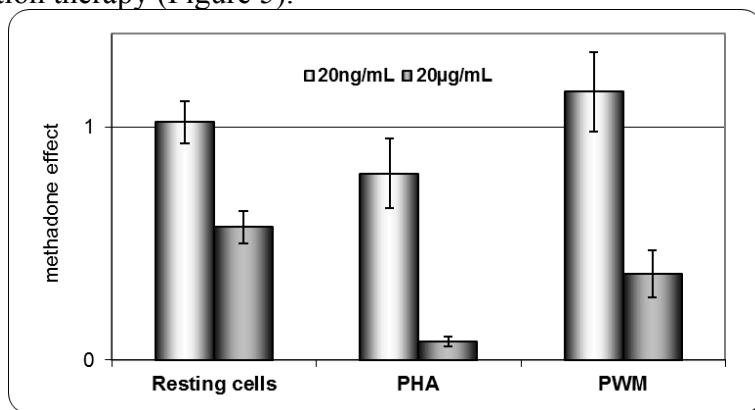


Figure 4

The *in vitro* effect of methadone on tritium-labeled uridine uptake by the resting, PHA- and PWM-activated lymphocytes isolated from heroin addicts (n=8).

For heroin addicts we found similar results with those obtained in the case of healthy subjects regarding the *in vitro* effect of methadone. Thus, the low concentration of methadone (20ng/mL) inhibited uridine uptake by PHA-activated T lymphocytes from heroin addicts, whilst having no conclusive effect on resting cells or PWM-activated B lymphocytes. A high concentration of methadone (20 μ g/mL) was immunosuppressive, especially for proliferating T lymphocytes. Results indicated that *in vivo* chronic challenge with heroin of μ opioid receptors on T lymphocytes did not alter their *in vitro* reactivity to methadone.

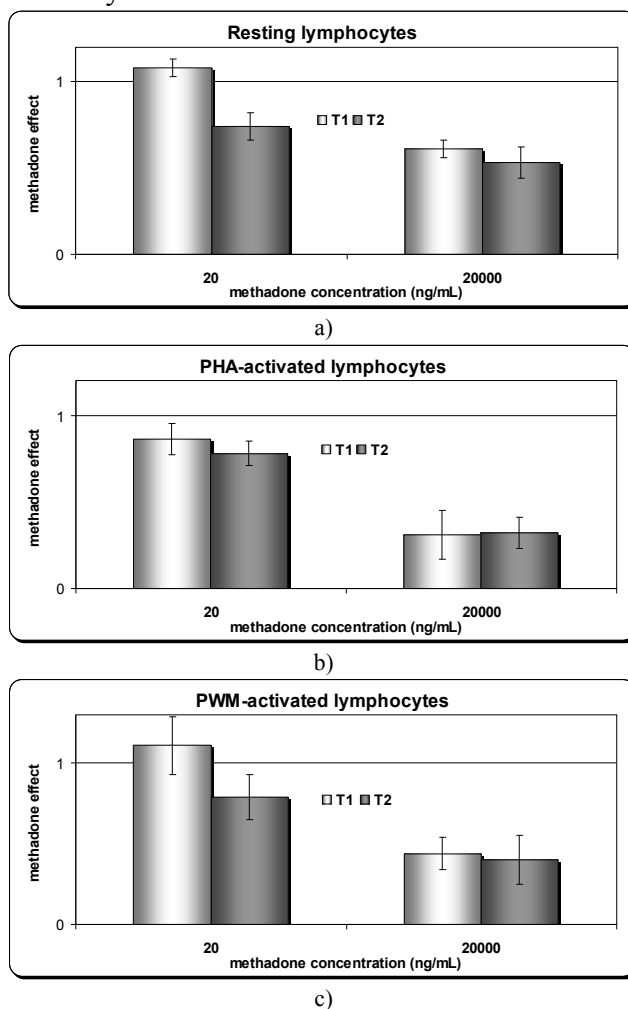


Figure 5

The *in vitro* effect of methadone on tritium-labeled uridine uptake by resting, PHA- and PWM-activated lymphocytes from heroin addicts before (T1) and 6 months (T2) after methadone replacement therapy onset.

Moreover, we showed that 6 months methadone substitution therapy did not alter the *in vitro* response of T lymphocytes to an additional pulse of methadone applied *ex vivo* (Figure 5b). Particularly in the case of patients on methadone therapy, an inhibitory action of low concentrations of methadone (20ng/mL) on resting and PWM-activated lymphocytes was noticed (Figure 5a,c), which did not manifest in the case of healthy subjects and heroin addicts (Figures 1,4). As such, the *in vivo* methadone challenge of lymphocytes made them more susceptible to the inhibitory effect exerted *in vitro* by low concentrations of methadone.

The inhibitory effect of low concentrations of methadone on activated T lymphocytes might be relevant in rheumatoid arthritis (RA), a systemic autoimmune disease characterized by chronic activation of pro-inflammatory immune cells [8]. We showed that 20ng/mL methadone inhibited uridine uptake by PHA-activated lymphocytes from RA patients, but also by lymphocytes which were not activated *ex vivo* (Figure 6).

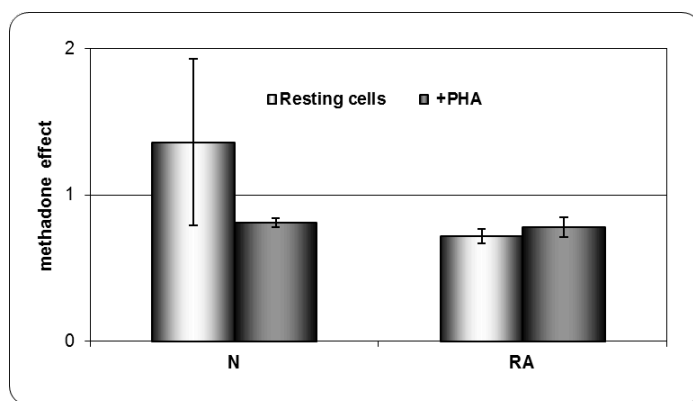


Figure 6

The *in vitro* effect of methadone on tritium-labeled uridine uptake by resting and PHA-activated lymphocytes from RA patients (n=5) vs. healthy subjects (N) (n=13).

We may presume that peripheral RA lymphocytes were in fact activated *in vivo* and were thus responsive to methadone challenge *in vitro*.

Conclusions

Our results indicated an *in vitro* stable immunomodulatory action of low concentrations of methadone (20ng/mL), exerted particularly on activated, proliferating T lymphocytes. In our experimental model we did not find desensitization of μ opioid receptors when challenged *in vivo* and/or *ex vivo* with methadone. Our findings might be relevant in reumatoid

arthritis disease aiming to control pain and inflammation simultaneously using opiates.

Acknowledgements

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