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Influence of stress and dexamethasone on plasma levels of antiepileptic drugs and theophylline in rats

Wpływ stresu oraz deksametazonu na poziom leków
przeciwpadaczkowych i teofiliny w osoczu szczurów

The ability of infection or inflammation to cause a reduction in the capacity of eliminating drugs from the plasma is a well-known fact. Bacterial endotoxin and other inflammatory agents are stressful stimuli that influence the hypothalamo-pituitary-adrenal axis [2]. Evidence shows that increased concentrations of endogenous glucocorticoids (GCs) appear consistently during stress and also their treatment with drugs in different diseases can modify the effectiveness and biotransformation of simultaneously used medications [1,2,4,10,11,13].

Cytochromes P-450 play a critical role in the metabolism of many endogenous and exogenous compounds, including steroid hormones, catalysing their oxidation. Cytochromes P450 (at least 21 forms) are a family of hemoproteins, abundant in the endoplasmatic reticulum of hepatocyte, each with a characteristic structure, substrate-binding affinities, and in some cases, differential regulatory responses to drugs or other xenobiotics. Because each cytochrome appears to reflect expression of a unique gene, standard nomenclature has been proposed based on dividing the P450 genes into families and subfamilies according to nucleotide sequence homology, i.e.: CYP3A; CYP2B1,2; CYP2C, CYP2C11 [10]. An important characteristic of some of the forms of cytochrome P-450 is that they are inducible [11,12]. It was demonstrated that a single administration of corticosterone was just enough to produce a statistically significant induction of biotransformation of substrates used [4]. It was also shown that cytochrome P-450 is inducible by synthetic GCs – dexamethasone (DEX) [10,11].

Since the amounts and types of cytochromes P450 in the liver may be rate-limiting for metabolism of foreign chemicals, enzyme induction may play an important role in such clinically relevant phenomena as interactions among therapeutic drugs, metabolic "idiosyncrasy" in hepatic drug reactions and interindividual differences in susceptibility to toxic effects of environmental chemicals [11].

With reference to the above, it was very interesting to investigate whether the pharmacokinetic of the selected drugs antiepileptic: phenytoin (PHT), valproate magnesium (VAL), phenobarbital

(PB) or theophylline (THEO) could be modified in the rats subjected to acute or chronic stress, and in those treated with DEX, too.

MATERIAL AND METHODS

All procedures were conducted according to NIH Animal Care and Use Committee guidelines and approved by the Ethics Committee of the Medical University of Lublin. The study was conducted on male Wistar rats (weighing initially 180-240 g). Animals were housed to a cage with food and water freely available, and maintained under a 12 h light-dark cycle (lights on at 8.00 a.m.). The following drugs were used intraperitoneally (ip): phenytoin (PHT) (13 mg/kg), valproate magnesium (VPL) (140 mg/kg), phenobarbital (PB) (18 mg/kg), theophylline (THEO) (50 mg/kg) and dexamethasone (DEX) (8 mg/kg).

Chronic unpredictable stress (CUS) procedure used was a variant of Katz et al. [3] method modified by Żebrowska-Łupina et al. [13]. Once a day the rats were subjected to the following kinds of unpredictable stressors: 20 s exposure to electric footshock (3mA, 0.2 s duration every 2 s), 2 h periods of immobilization at 20^0 C or at 4^0 C, 5 min exposure to electric bell, 3 min periods of swimming in cold water (12^0 C) or 5 min periods of illumination (80$\pm$klx) and 48 h periods of food deprivation. Each stressor was repeated 2 times during the 16-day period. All antiepileptic drugs and theophylline were injected at a single dose 48 h or 1 h after the last stress session of the chronic unpredictable stress (CUS) (groups of animals: II and III, respectively).

Acute stress was an electric footshock (3 mA/0.2s/2s x 10) applied for 20s. 1 h later, all the drugs: antiepileptics and theophylline were injected (group IV). Additionally, an experiment was conducted on the rats that were first treated with DEX at a single dose of 8 mg/kg and then 3 h later with all antiepileptic drugs and theopylline (group V). Control group of animals (group I – no stress) was treated with all antiepileptic drugs alone and theophylline. 30 (VLP and THEO), 60 (PB) or 120 min (PHT) later the rats were killed by decapitation and their blood was taken to estimate the level of drugs. The blood samples were centrifuged at 10 000 rpm for 5 min. The obtained plasma levels were carried out by immunofluorescence, with the use of an Abbott TDx analyzer (Abbott, Irving, TX, USA). In order to measure the free fraction of the antileptics and the total level of THEO, the rest of the plasma was pipetted into Microton-30 microconcentrators (Amicon, Inc., Beverly, MA, USA) and again centrifuged (at 10 000 rpm for 5 min), then transferred into Abbott TDx system. Total THEO and free plasma levels (other drugs) were expressed in ng/ml as means$\pm$ SEM.

Plasma levels of drugs were assessed by one-way analysis of variance (ANOVA) and Tukey-Kramer parametric post test.

RESULTS

In the rats subjected to both chronic stress and acute stress, a significant decrease of the free plasma levels of PHT and VPL was observed (ANOVA: $F4.25=8.032$, $p<0.0003$ and $F4.35=11.659$, $p<0.0001$ respectively). Instead, DEX evoked an irrelevant decrease of the free plasma levels of PHT and VPL in the rats (Fig. 1 and Fig. 2).

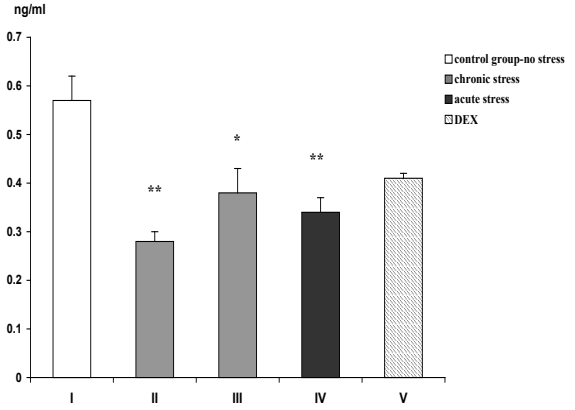

Fig. 1. The effect of stress or DEX on plasma level of PHT in rats. PHT was administered ip, at single dose of 13 mg/kg to the all groups of rats; I – control group (no stress), II group – 48 h after the last session of chronic stress, III group – 1 h after the last session of chronic stress, IV group – 1 h after acute stress and V group – rats treated of DEX. Tukey–Kramer parametric post test, **p<0.02 and *p<0.05 vs I group. N=6-8

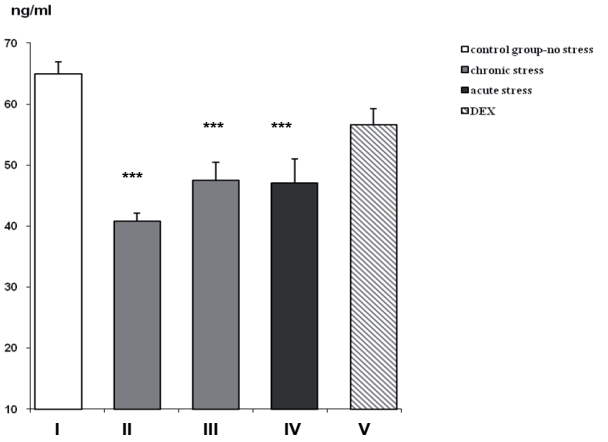

Fig. 2. The effect of stress or DEX on plasma level of VPL in rats. VPL was administered ip, at a single dose of 140 mg/kg to the all group of rats; I – control group (no stress), II group – 48 h after the last session of chronic stress, III group – 1 h after the last session of chronic stress, IV group – 1 h after acute stress and V group – rats treated of DEX. Tukey-Kramer parametric post test, ***p<0.001 vs I group. N=6-8

Neither in the rats submitted to chronic stress nor in those subjected to acute stress the free plasma level of PB was changed, but a significant increase of the free plasma level was observed in the rats injected with DEX (ANOVA: $F_{5.36}=13.98$, $p<0.0001$) (Fig. 3).

In the rats submitted to chronic stress the total plasma level of THEO did not change, but a significant decrease of the total plasma level of THEO in the rats with the acute stress or treated with DEX was observed (ANOVA: $F_{4.32}$=14.495, p<0.0001) (Fig. 4).

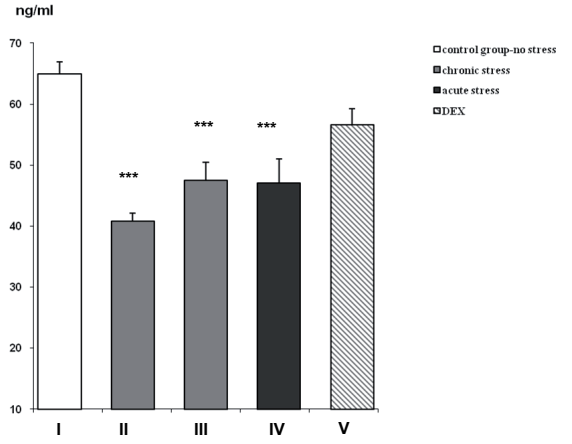

Fig. 3. The effect of stress or dexamethasone (DEX) on plasma level of PB in rats. PB was administered ip, at a single dose of 18 mg/kg to the all group of rats; I – control group (no stress), II group – 48 h after the last session of chronic stress, III group – 1 h after the last session of chronic stress, IV group – 1 h after acute stress and V group – rats treated of DEX. Tukey-Kramer parametric post test, **p<0.01 vs I group. N=6-8

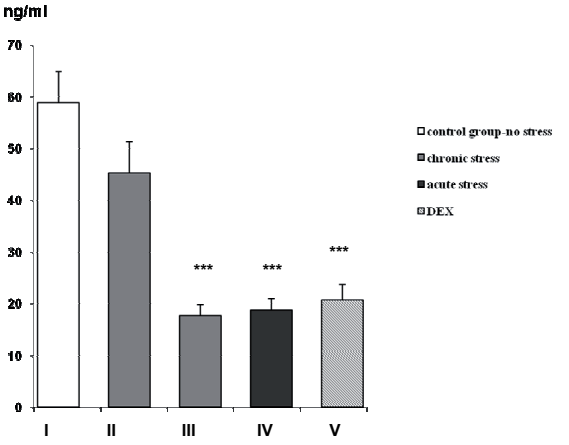

Fig. 4. The effect of stress or DEX on plasma level of THEO in rats. THEO was administered ip, at a single dose of 50 mg/kg to the all group of rats; I – control group (no stress), II group – 48 h after the last session of chronic stress, III group – 1 h after the last session of chronic stress, IV group – 1 h after acute stress and V group – rats treated of DEX. Tukey-Kramer parametric post test, ***p<0.001 vs group I. N=6-8

DISCUSSION

This study clearly showed that acute or chronic stress as well as DEX changed plasma levels of the drugs used simultaneously. Our previous study (not published yet) revealed that in the rats subjected to chronic unpredictable stress procedure (in this experiment the same stress occurs) the levels of serum corticosterone were elevated. Moreover, other research showed that administration of toxic agents to rats leads to elevated corticosterone plasma levels [4]. It was indicated that both endogenous corticosterone and synthethic DEX could induce or suppress different forms of cytochrome P450 (especially CYP2C11 or CYP3A), depending on the dose and time of their activity. Iber et al. [2] demonstrated that the response to DEX in the experiment with cultured rat hepatocytes was dependent on the dose: low concentrations of DEX induced and higher concentrations suppressed the expression of CYP2C11. To determine the specificity of the effect, they tested the physiological GCs-corticosterone at various concentrations also and a similar bimodal effect was seen. Additionally, they demonstrated that both phases of induction and suppression of the CYP2C11 are dependent on the glucocorticoid receptor and this expression might be highly sensitive to changes in GCs secretion.

On the other hand, Liddle et al. [5] reported that long-term treatment of hepatocyte cultures with DEX at the dose that should suppress the CYP2C11 caused an increase in its expression. Moreover, a significant increase in the concentration of HLp (a form of human liver cytochrome P450) was observed in the patients who received DEX, PB and diphenylhydantoin [11]. In our study, we observed a significant decrease in the plasma levels of PHT and VPL or THEO in rats submitted to stress and also in rats treated with DEX, which might indicate induction of different forms of cytochrome P450 in the liver.

Cytochrome P450 2C (CYP2C) plays an important role in PHT metabolism. This antiepileptic drug is known to be substrate as well as inducer of cytochrome P450 in the mammalian liver (in rats CYP2B1, 2) both in the central and peripherial neurons system [9]. This effect of PHT can cause occurrences of severer gingival overgrowth (a side effect of phenytoin) in some patients, exhibited significantly higher serum PHT concentration, indicating that its metabolism is an important determinant for the severity of the disease [7]. Moreover, the competition between both inducers/substrates could therefore explain the difficulties in the therapy of epileptic patients of PHT especially with the incorporated drug [7].

Valproic acid (VPA), a major antiepileptic drug against several types of epileptic seizures, has recently been also used in bipolar disorders, migraine or neuropathic pain. Valproate undergoes metabolism by a variety of conjugation and oxidative processes. In particular, research focused on the involvement of the P450-dependent terminal desaturation metabolite 4-eneVPA and its β-oxidation metabolite (E)-2,4-diene VPA. Although the desaturation pathway is relatively minor, an elevated level of 4-ene VPA was observed, especially by coadministration of a P450-inducing anticonvulsant, such as PHT, PB and carbamazepine, which is a known risk factor for VPA-induced hepatotoxicity [8].

THEO, a nonselective phosphodiesterase inhibitor, is used for treatment of asthma and COPD (chronic obstructive pulmonary disease). The reduced drug clearance that accompanies infection or inflammation can result in elevated plasma drug concentration in individuals after a standard dose.

For drugs with a low therapeutic index, this can result in drug toxicity, as was seen in children with influenza A infections who took theophylline for their asthma [2].

However, we observed different results with PB in our experiment. The levels of the drug in the plasma of rats subjected to stress and DEX were increased. Explanation of this phenomenon may be connected to cytochrome P450 and its expression and also the endogenous inducer, which is likely to be a steroid. PB is a substrate for some P450 enzymes of family II that are also highly active in steroid hydroxylation. It is plausible that PB competitively inhibits an enzyme that metabolizes an endogenous steroidal substrate, thus leading to its intracellular accumulation [6]. Moreover, another study demonstrated that PB and DEX may also induce the same cytochrome-CYP2C6 [6, 11], but the response to DEX is rapid, whereas induction by PB occurs slowly after 8–10 h lag [6].

In our experiment, plasma level of PB was increased, which proves lower metabolism of that drug in the rats subjected to acute stress, especially after DEX when the increase was statistically significant. We may conclude that accumulation of steroidal substrate can cause suppression of the enzymes that metabolize PB, although this effect is not so obvious and needs further explanation.

The PB induction phenomenon is of more general interest because the drug also induces the expression of $mRNA_s$ encoding other enzymes that participate in drug detoxication, epoxide hydroxylase UDP-glucoronosyltransferase, and glutathione-s-transferase [6].

On the other hand, the role of augmented resistance to drugs after administration of corticosterone to rats and decreased plasma levels of the toxic agents was emphasized through an increased ability of the liver for drug biotransformation. However, it is known that glucocorticoids increase tissue tolerance to toxic agents without any increase of the drug biotransformation [4].

Knowledge on the subject of enzymatic expression concerning biotransformation of drugs can successfully be used in the treatment of many diseases and in co-administration of various medications. Such environmental factors as for example, stress should also be taken into consideration while using pharmacotherapy. Further studies are necessary to elucidate this issue.

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SUMMARY

Elevation of plasma glucocorticoids (GCs) during stress and GCs treatment in different diseases can modify the liver enzymes activity, biotransformation and the effectiveness simultaneously used medications. Recently the effect of GCs, especially of dexamethasone (DEX) – a synthetic GCs receptor agonist on cytochrome P450 enzymes was described. The aim of the present study was to investigate whether the pharmacokinetc of the selected antiepileptic drugs: phenytoin (PHT), vaproate magnesium (VPL), phenobarbital (PB) or theophylline (THEO) could be modified in the rats subjected to acute or chronic stress, and in those treated with DEX, too. All drugs were injected at a single dose 48 h or 1 h after the last stress session of the chronic unpredictable stress (a variant of Katz et al.). Acute stress was an electric footshock. 1 h later all drugs were injected. Additionally, an experiment was conducted on the rats that were first treated with DEX at a single dose and then 3 h later with all drugs. The control group of animals was treated with drugs alone. 30 (VLP and THEO), 60 (PB) or 120 min (PHT) later, the blood of rats was taken to estimate the level of drugs by immunofluorescence. In the rats subjected to chronic and acute stress and also after DEX treated a decrease of the free plasma levels of PHT and VPL was observed. The total plasma level of THEO was significantly decreased in rats with only acute stress or treated with DEX. However, the free plasma level of PB was significantly increased in rats injected with DEX. These date obviously indicate that each kind of stress as well as GCs (e.g. DEX) may change the metabolism of drugs and it should be taken into consideration during pharmacotherapy.

STRESZCZENIE

Zwiększone wydzielanie oraz podniesiony poziom endogennych glikokortykosteroidów (GCs) we krwi podczas stresu i stosowanie różnych preparatów GCs w wielu chorobach może modulować aktywność enzymów w wątrobie, biotransformację i efektywność innych równocześnie stosowanych leków. W ostatnich latach wykazano wpływ GCs oraz deksametazonu (DEX), syntetycznego agonisty receptorów GCs, na enzymy cytochromu P450. Celem pracy było zbadanie, czy farmakokinetyka wybranych leków przeciwpadaczkowych: fenytoiny (PHT), walproinianu magnezu (VAL) i fenobarbitu (PB) oraz teofiliny (THEO) może być zmieniona u szczurów poddanych działaniu ostrego i przewlekłego stresu, jak również poddanych działaniu DEX. Wszystkie leki były wstrzykiwane w jednorazowej dawce 48 godzinę po ostatniej sesji przewlekłego stresu (procedura wg Katz i wsp.). Stresem ostrym było zastosowanie jednorazowo podłogi elektrycznej. Godzinę później były wstrzykiwane wszystkie badane leki. Dodatkowo przeprowadzono eksperyment z DEX, podanym dootrzewnowo w jednorazowej dawce, a następnie po 3 godzinach wstrzykiwano wszystkie badane leki. Zwierzęta kontrolne otrzymywały tylko badane leki. 30, 60 i 120 minut później pobierano krew do oznaczeń poziomu leków metodą immunofluorescencyjną. Przeprowadzone badania wykazały, że u szczurów poddanych działaniu stresu oraz DEX ulega obniżeniu wolna frakcja PHT i VAL w surowicy oraz całkowite stężenie teofiliny. Obserwowano natomiast znaczne podwyższenie wolnej frakcji PB w surowicy po zastosowanym wcześniej DEX. Uzyskane wyniki dowodzą, że każdy rodzaj stresu oraz GCs, jak np. DEX, może zmienić metabolizm leków, co powinno być rozważane podczas farmakoterapii.